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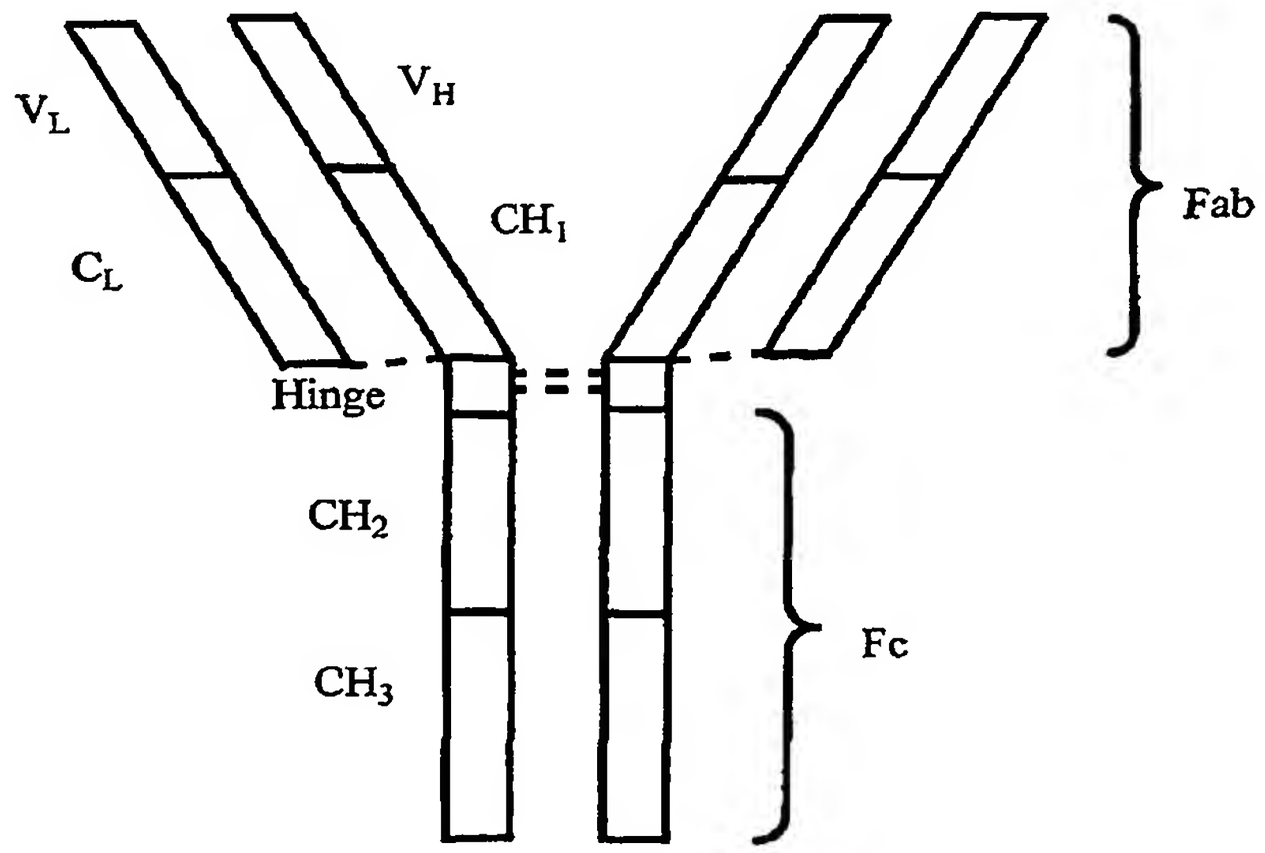
(54) Title: ANTIGEN-BINDING PROTEINS TARGETING S. AUREUS ORF0657N



(57) Abstract: The present invention features antigen binding protein that bind an ORF0657n target region (SEQ ID NO: 1). ORF0657n is an *S. aureus* protein. ORF0657n target regions are provided by the mAb 1G3.BD4, mAb 2H2.BE11, mAb 13C7.BC1, and mAb 13G11.BF3 binding sites. In a lethal model challenge, mAb 2H2.BE11 and mAb 13C7.BC1 provided for increased survival against *S. aureus* infection. There was also protection demonstrated in an *ex vivo* model with either the IgG1 or the IgG2b form of mAb 2H2; and in a passive immunization murine indwelling catheter model using mAb 2H2.BE11.

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TITLE OF THE INVENTION

ANTIGEN-BINDING PROTEINS TARGETING S. AUREUS ORF0657n

RELATED APPLICATIONS

The present application claims priority to U.S. Provisional Application No. 60/763,023, filed January 27, 2006, which is hereby incorporated by reference herein.

BACKGROUND OF THE INVENTION

The references cited throughout the present application are not admitted to be prior art to the claimed invention.

Staphylococcus aureus is a pathogen responsible for a wide range of diseases and conditions. Examples of diseases and conditions caused by *S. aureus* include bacteremia, infective endocarditis, folliculitis, furuncle, carbuncle, impetigo, bullous impetigo, cellulitis, botryomyosis, toxic shock syndrome, scalded skin syndrome, central nervous system infections, infective and inflammatory eye disease, osteomyelitis and other infections of joints and bones, and respiratory tract infections. (*The Staphylococci in Human Disease*, Crossley and Archer (eds.), Churchill Livingstone Inc. 1997.)

Immunological based strategies can be employed to control *S. aureus* infections and the spread of *S. aureus*. Immunological based strategies include passive and active immunization. Passive immunization employs immunoglobulins targeting *S. aureus*. Active immunization induces immune responses against *S. aureus*.

SUMMARY OF THE INVENTION

The present invention features antigen binding protein that bind an ORF0657n target region (SEQ ID NO: 1). ORF0657n is an *S. aureus* protein. ORF0657n target regions are provided by the mAb 1G3.BD4, mAb 2H2.BE11, mAb 13C7.BC1, and mAb 13G11.BF3 binding sites. In a lethal model challenge, mAb 2H2.BE11 and mAb 13C7.BC1 provided for increased survival against *S. aureus* infection. There was also protection demonstrated in an *ex vivo* model with either the IgG1 or the IgG2b form of mAb 2H2; and in a passive immunization murine indwelling catheter model using mAb 2H2.BE11.

Mouse hybridoma cell lines producing mAb 1G3.BD4, mAb 2H2.BE11; mAb 13C7.BC1, and mAb 13G11.BF3 were deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, in accordance with Budapest Treaty on September 30, 2005. The cells lines were designated: ATCC No. PTA-7124 (producing mAb 2H2.BE11), ATCC No. PTA-7125 (producing mAb 13C7.BC1), ATCC No. PTA-7126 (producing mAb 1G3.BD4), and ATCC No. PTA-7127 (producing mAb 13G11.BF3).

Thus, a first aspect of the present invention features an isolated antigen binding protein comprising a first variable region and a second variable region. The first and second variable regions

bind one or more target regions selected from the group consisting of: mAb 1G3.BD4 target region, mAb 2H2.BE11 target region, mAb 13C7.BC1 target region, and mAb 13G11.BF3 target region.

Reference to "isolated" indicates a different form than found in nature. The different form can be, for example, a different purity than found in nature and/or a structure that is not found in nature. A structure not found in nature includes recombinant structures where different regions are combined together, for example, humanized antibodies where one or more murine complementary determining regions is inserted onto a human framework scaffold or a murine antibody is resurfaced to resemble the surface residues of a human antibody, hybrid antibodies where one or more complementary determining regions from an antigen binding protein is inserted into a different framework scaffold, and antibodies derived from natural human sequences where genes coding for light and heavy variable domains were randomly combined together.

The isolated protein is preferably substantially free of serum proteins. A protein substantially free of serum proteins is present in an environment lacking most or all serum proteins.

A "variable region" has the structure of an antibody variable region from a heavy or light chain. Antibody heavy and light chain variable regions contain three complementary determining regions interspaced onto a framework. The complementary determining regions are primarily responsible for recognizing a particular epitope.

A target region is defined with respect to the ORF0657n region (SEQ ID NO: 1) bound by mAb 1G3.BD4, mAb 2H2.BE11, mAb 13C7.BC1, or mAb 13G11.BF3. For example, the mAb IG3.BD4 target region is the ORF0657n region to which mAb IG3.BD4 binds.

A protein binding an identified target region competes with either mAb 1G3.BD4, mAb 2H2.BE11, mAb 13C7.BC1, or mAb 13G11.BF3 for binding to the target region. For example, a protein competing with mAb 1G3.BD4 binding to ORF0657n binds to the mAb 1G3.BD4 target region.

A protein that competes with either the monoclonal antibody mAb 1G3.B3, mAb 2H2.B8, mAb 13C7.D12, or mAb 13G11.C11 reduces binding of the monoclonal antibody to ORF0657n by at least about 20%, preferably at least about 50%, when excess and equal amounts of the competing protein and monoclonal antibody are employed.

Reference to "protein" indicates a contiguous amino acid sequence and does not provide a minimum or maximum size limitation. One or more amino acids present in the protein may contain a post-translational modification, such as glycosylation or disulfide bond formation.

A preferred antigen binding protein is a monoclonal antibody. Reference to a "monoclonal antibody" indicates a collection of antibodies having the same, or substantially the same, complementary determining region, and binding specificity. The variation in the antibodies is that which would occur if the antibodies were produced from the same construct(s).

Monoclonal antibodies can be produced, for example, from a particular hybridoma and from a recombinant cell containing one or more recombinant genes encoding the antibody. The antibody

may be encoded by more than one recombinant gene where, for example, one gene encodes the heavy chain and one gene encodes the light chain.

Another aspect of the present invention describes a nucleic acid containing a recombinant gene comprising a nucleotide sequence encoding an antibody variable region. The antibody variable region can bind a target region selected from the group consisting of: mAb IG3.BD4 target region, mAb 2H2.BE11 target region, mAb 13C7.BC1, and mAb 13G11.BF3 target region.

A recombinant gene contains recombinant nucleic acid encoding a protein along with regulatory elements for proper transcription and processing (which may include translational and post translational elements). The recombinant nucleic acid by virtue of its sequence and/or form does not occur in nature. Examples of recombinant nucleic acid include purified nucleic acid, two or more nucleic acid regions combined together providing a different nucleic acid than found in nature, and the absence of one or more nucleic acid regions (*e.g.*, upstream or downstream regions) that are naturally associated with each other.

Another aspect of the present invention describes a recombinant cell comprising one or more recombinant genes encoding an antibody variable region that binds to a target region selected from the group consisting of: mAb IG3.BD4 target region, mAb 2H2.BE11 target region, mAb 13C7.BC1, and mAb 13G11.BF3 target region. Multiple recombinant genes are useful, for example, where one gene encodes an antibody heavy chain or fragment thereof containing the V_h region and another nucleic acid encodes an antibody light chain or fragment thereof containing the V_l region.

Another aspect of the present invention comprises a method of producing a protein comprising an antibody variable region. The method comprising the steps of: (a) growing a recombinant cell comprising recombinant nucleotide acid encoding for a protein under conditions wherein the protein is expressed; and (b) purifying the protein.

Another aspect of the present invention describes a pharmaceutical composition. The composition contains a therapeutically effective amount of an antigen binding protein and a pharmaceutically acceptable carrier.

A therapeutically effective amount is an amount sufficient to provide a useful therapeutic or prophylactic effect. For a patient infected with *S. aureus*, an effective amount is sufficient to achieve one or more of the following effects: reduce the ability of *S. aureus* to propagate in the patient or reduce the amount of *S. aureus* in the patient. For a patient not infected with *S. aureus*, an effective amount is sufficient to achieve one or more of the following: a reduced susceptibility to *S. aureus* infection or a reduced ability of the infecting bacterium to establish persistent infection for chronic disease.

Another aspect of the present invention describes a method of detecting the presence of an OFR0657n antigen in a solution or on a cell. The method involves providing a binding protein described herein to the solution or cell and measuring the ability of the binding protein to bind to the antigen in the solution or cell. Measurements can be quantitative or qualitative.

Reference to ORF0657n antigen includes full-length ORF0657n or a derivative thereof having an epitope that is recognized by mAb 1G3.B3, mAb 2H2.B8, mAb 13C7.D12, or mAb 13G11.C11. Examples of derivatives include truncated versions; and full-length or truncated versions of ORF0657n containing one or more of the following amino acid alterations: one or more additions, one or more substitutions, and one or more deletions.

Another aspect of the present invention features a method of treating a patient against a *S. aureus* infection. The method comprises the step of administering to the patient an effective amount of an antigen binding protein described herein. The patient being treated may, or may not, be infected with *S. aureus*. Preferably, the patient is a human.

Another aspect of the present invention describes a cell line producing a protein that is either mAb 1G3.B3, mAb 2H2.B8, mAb 13C7.D12, or mAb 13G11.C11, or that competes with either mAb IG3.B3, mAb 2H2.B8, mAb 13C7.D12, or mAb 13G11.C11 for binding to ORF0657n. Preferred cells lines are hybridomas, and recombinant cell lines containing recombinant nucleic acid encoding the protein.

Reference to open-ended terms such as "comprises" allows for additional elements or steps. Occasionally phrases such as "one or more" are used with or without open-ended terms to highlight the possibility of additional elements or steps.

Unless explicitly stated reference to terms such as "a" or "an" is not limited to one. For example, "a cell" does not exclude "cells". Occasionally phrases such as one or more are used to highlight the possible presence of a plurality.

Other features and advantages of the present invention are apparent from the additional descriptions provided herein including the different examples. The provided examples illustrate different components and methodology useful in practicing the present invention. The examples do not limit the claimed invention. Based on the present disclosure the skilled artisan can identify and employ other components and methodology useful for practicing the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the structure of an IgG molecule. "V_L" refers to a light chain variable region. "V_H" refers to a heavy chain variable region. "C_L" refers to a light chain constant region. "CH_1", "CH_2" and "CH_3" are heavy chain constant regions. Dashed lines indicate disulfide bonds.

Figure 2 illustrates a matrix outlining the reactivities of different monoclonal antibodies in a pair-wise binding study. The panel of monoclonal antibodies fell into three reactive areas by the BIACORE® method.

Figures 3A-3C: Groups of BALB/c mice (n = 20) were treated 20 hours prior to bacterial challenge with an i.p. injection of: ■, mAb 13C7.BC1; □, mAb 6G6.A8 (isotype control); or ○, PBS. Mice were challenged with *S. aureus* by i.v. injection and survival was monitored. Fig. 3A-

0.49 mg mAb 13C7.BC1; 0.45 mg mAb 6G6.A8; and 9.8 x 10^8 CFU *S. aureus* Becker. Fig. 3B- 0.49 mg mAb 13C7.BC1; 0.45 mg mAb 6G6.A8; and 9.6 x 10^8 CFU *S. aureus* Becker. Fig. 3C- 0.50 mg mAb13C7.BC1; 0.45 mg mAb 6G6; and 9.9 x 10^8 CFU *S. aureus* Becker.

Figures 4A and 4B: Groups of BALB/c mice (n = 20) were treated 20 hours prior to bacterial challenge with an i.p. injection of: ■, mAb 13C7.BC1 (0.5 mg); □, mAb 6G6.A8 (isotype control) (0.5 mg); or ○, PBS (0.5 ml). Mice were challenged with *S. aureus* by i.v. injection and survival was monitored. Fig. 4A illustrates results with 2.09 X 10^8 CFU *S. aureus* UK58. Fig. 4B illustrates results with 2.15 X 10^8 *S. aureus* UK 58.

Figures 5A-5C: Groups of BALB/c mice (n = 20) were treated 20 hours prior to bacterial challenge with an i.p. injection of: ■, mAb 2H2.BE11, □, mAb 6G6.A8 (isotype control); ○, PBS. Mice were challenged with *S. aureus* by i.v. injection and survival was monitored. Fig. 5A- 0.43 mg mAb 2H2.BE11; 0.5 mg mAb 6G6.A8; and 9.8 x 10^8 CFU *S. aureus* Becker. Fig. 5B- 0.43 mg mAb 2H2.BE11; 0.5 mg mAb 6G6.A8; and 8.3 x 10^8 CFU *S. aureus* Becker. Fig. 5C- 0.43 mg mAb 2H2.BE11; 0.5 mg mAb 6G6.A8; and 9.3 x 10^8 CFU *S. aureus* Becker.

DETAILED DESCRIPTION OF THE INVENTION

ORF0657n is an *S. aureus* protein located at the *S. aureus* outer membrane. ORF0657n has been found to be well conserved in different strains of *S. aureus*. (Anderson *et al.*, International Publication No. WO 2005/009379, International Publication Date February 3, 2005.) Different ORF0657n derivatives can be used to produce a protective immune response against *S. aureus* infection. (Anderson *et al.*, International Publication No. WO 2005/009379, International Publication Date February 3, 2005.)

Due to their ability to recognize ORF0657n, the antigen binding proteins described herein can be used, for example, as a tool in the production, characterization, or study of ORF0657n based antigens. Antigen binding protein recognizing appropriate ORF0657n epitopes can also be used agent to treat *S. aureus* infection.

I. Antigen Binding Protein

Antigen binding proteins contain an antibody variable region providing for specific binding to an epitope. The antibody variable region can be present in, for example, a complete antibody, an antibody fragment, and a recombinant derivative of an antibody or antibody fragment.

Different classes of antibodies have different structures. Different antibody regions can be illustrated by reference to IgG (Figure 1). An IgG molecule contains four amino acid chains: two longer length heavy chains and two shorter light chains. The heavy and light chains each contain a constant region and a variable region. Within the variable regions are three hypervariable regions responsible for antigen specificity. (See, for example, Breitling *et al.*, Recombinant Antibodies, John Wiley & Sons, Inc. and Spektrum Akademischer Verlag, 1999; and Lewin, Genes IV, Oxford University Press and Cell Press; 1990.)

The hypervariable regions (also referred to as complementarity determining regions), are interposed between more conserved flanking regions (also referred to as framework regions). Amino acids associated with framework regions and complementarity determining regions can be numbered and aligned as described by Kabat *et al.*, Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services, 1991.

The two heavy chain carboxyl regions are constant regions joined by disulfide binding to produce an Fc region. The Fc region is important for providing antibody biological activity such as complement and macrophage activation. Each of the two heavy chains making up the Fc region extend into different Fab regions through a hinge region.

In higher vertebrates there are two classes of light chains and five classes of heavy chains. The light chains are either κ or λ. The heavy chains define the antibody class and are either α, δ, ε, γ, or μ. For example, IgG has a γ heavy chain. Subclasses also exist for different types of heavy chains such as human γ_1, γ_2, γ_3, and γ_4. Heavy chains impart a distinctive conformation to hinge and tail regions. (Lewin, Genes IV, Oxford University Press and Cell Press, 1990.)

Antibody fragments containing an antibody variable region include Fv, Fab, and Fab_2 regions. Each Fab region contains a light chain made up of a variable region and a constant region, and a heavy chain region containing a variable region and a constant region. A light chain is joined to a heavy chain by disulfide bonding through constant regions. The light and heavy chain variable regions of a Fab region provide for an Fv region that participates in antigen binding.

The antibody variable region can be present in a recombinant derivative. Examples of recombinant derivatives include single-chain antibodies, diabody, triabody, tetrabody, and miniantibody. (Kipriyanov *et al, Molecular Biotechnology 26*:39-60, 2004.)

The antigen binding protein can contain one or more variable regions recognizing the same or different epitopes. (Kipriyanov *et al., Molecular Biotechnology 26*:39-60, 2004.)

II. Generation of Antigen Binding Protein Directed to an Identified Target Region

Different antigen binding proteins directed to the mAb 1G3.BD4 target region, mAb 2H2.BE11 target region, mAb 13C7.BC1 target region, or mAb 13G11.BF3 target region can be generated starting with the respective monoclonal antibody. Alternatively, the epitope recognized by a binding protein can be used to select additional binding proteins.

The mAb 2H2.BE11 target region appears to be located at approximately amino acids 76-357 of ORF0657n. A polypeptide containing amino acids 76-357 of ORF0657n, or a full-length ORF0657n, can be used as a target antigen to select for antibodies. The target region of the generated antibodies can be determined.

A variety of techniques are available to select for a protein recognizing an antigen. Examples of such techniques include use of phage display technology and hybridoma production. Human antibodies can be produced using chimeric mice such as a XenoMouse or Trans-Chromo mouse.

(*E.g.*, Azzazy *et al., Clinical Biochemistry 35*:425-445, 2002, Berger *et al., Am. J. Med. Sci. 324(1)*:14-40, 2002.)

The monoclonal antibodies mAb 1G3.BD4, mAb 2H2.BE11, mAb 13C7.BC1, and mAb 13G11.BF3 contain variable regions recognizing ORF0675n. Additional binding proteins recognizing ORF0657n can be produced based on antibody variable regions. Additional binding proteins can, for example, be produced by modifying an existing monoclonal antibody and by using variable region sequence information. Protein construction and sequence manipulation can be performed using recombinant nucleic acid techniques.

The monoclonal antibodies mAb 1G3.BD4, mAb 2H2.BE11, mAb 13C7.BC1, and mAb 13G11.BF3 are murine antibodies. For human therapeutic applications, preferred binding proteins based on such mAb's are designed to reduce the potential generation of human anti-mouse antibodies recognizing the murine regions.

The potential generation of human anti-mouse antibodies can be reduced using techniques such as murine antibody humanization, de-immunization, and chimeric antibody production. (See, for example, O'Brien *et al.*, Humanization of Monoclonal Antibodies by CDR Grafting, p 81-100, From *Methods* in Molecular Biology Vol. 207: Recombinant antibodies for Cancer Therapy: Methods and Protocols (Eds. Welschof and Krauss) Humana Press, Totowa, New Jersey, 2003; Kipriyanov *et al., Molecular Biotechnology 26*:39-60, 2004; Gonzales *et al., Tumor Biol. 26*:31-43, 2005, Presta, *Advanced Drug Delivery Reviews 58*:640-656, 2006, Tsurushita *et al., Methods 36*:69-83, 2005, Roque *et al., Biotechnol. Prog. 20*:639-654, 2004.)

Murine antibodies can be humanized using techniques such as grafting complementary determining regions into a framework region or resurfacing. Resurfacing (also known as veneering) involves modifying a variable region so the surface exposed regions are humanized.

Grafting complementary determining regions involves taking such regions or a portion of such regions from, for example, a murine source and inserting the regions into a human variable region framework. The human framework used for grafting can be selected based on sequence homology to the variable region *(e.g.*, murine) from which the region was obtained. Essential framework residues associated with grafted complementary determining regions should also be provided in the new framework.

De-immunization involves altering potential linear T-cell epitopes present in the antibody. The epitopes can be identified based on a bioinformatics scan of know human HLA class I and/or class II epitopes. (Presta, *Advanced Drug Delivery Reviews 58*:640-656, 2006.)

A chimeric antibody contains a human constant region along with a variable region from a different organism, such as a mouse. The human constant region provides an Fc region.

Additional examples of alterations include providing a variable region in, for example, a single chain antibody, a diabody, a triabody, a tetrabody, and a miniantibody. (Kipriyanov *et al., Molecular Biotechnology 26*:39-60, 2004.) The antigen binding protein can contain one or more variable

regions recognizing the same or different epitopes. (*Id.*) Additional embodiments of the present invention are directed to a single chain antibody, a diabody, a triabody, a tetrabody, or a miniantibody directed to the mAb 1G3.BD4, mAb 2H2.BE11, mAb 13C7.BC1, or mAb 13G11.BF3 binding site.

III. Binding Protein Directed to the mAb 2H2.BE11 Target Region

As described in the Examples provided below, the mAb 2H2.BE11 target region was further characterized and the amino acids sequence of the variable regions was determined. The identified target region and the sequence information facilitate obtaining different binding proteins directed to the mAb 2H2.BE11 target region.

In an embodiment of the present invention, the binding protein binds to a polypeptide consisting of amino acids 76-357 of SEQ ID NO: 1. Preferably, the binding protein is either a human antibody, a humanized antibody, a de-immunized antibody, or chimeric antibody. Preferred antibodies are isolated antibodies and monoclonal antibodies.

The amino acids sequences of the mAb 2H2.BE11 variable regions are provided by SEQ ID NO: 20 (V_h) and SEQ ID NO: 21 (V_l). The complementary determining regions (CDR's) within V_h were identified at amino acids 36-45, 50-65, and 98-107. The CDR's within V_l were identified at amino acids 24-33, 49-55, and 88-96 of SEQ ID NO: 21.

In different embodiments directed to a V_h region, the binding protein binds the mAb 2H2.BE11 target region and comprises, consists, or consists essentially of: a first V_h CDR comprising, consisting, or consisting essentially of amino acids 36-45 of SEQ ID NO: 20 or a sequence differing from amino acids 36-45 by one amino acid; a second V_h CDR comprising, consisting, or consisting essentially of amino acids 50-65 of SEQ ID NO: 20 or a sequence differing from amino acids 50-65 by one amino acid; and a third V_h CDR comprising, consisting, or consisting essentially of amino acids 98-107 of SEQ ID NO: 20 or a sequence differing from amino acids 98-107 by one amino acid.

In different embodiments directed to a V_l region, the binding protein binds the mAb 2H2.BE11 target region and comprises, consists, or consists essentially of a first V_l CDR comprising, consisting, or consisting essentially of amino acids 24-33 of SEQ ID NO: 21 or a sequence differing from amino acids 24-33 by one amino acid; a second V_l CDR comprising, consisting, or consisting essentially of amino acids 49-55 of SEQ ID NO: 21 or a sequence differing from amino acids 49-55 by one amino acid; and a third V_l CDR comprising, consisting, or consisting essentially of amino acids 88-96 of SEQ ID NO: 21 or a sequence differing from amino acids 88-96 by one amino acid.

Reference to "consisting essentially of" with respect to a variable region, CDR region, or antibody sequence, indicates the possible presence of one or more additional amino acids, where such amino acids do not significantly decrease binding to the target.

An amino acid difference can be an amino acid deletion, insertion, or substitution. In substituting amino acids to maintain activity, the substituted amino acids should have one or more similar properties such as approximately the same charge, size, polarity and/or hydrophobicity.

Preferably, an amino acid substitution is a conservative substitution. A conservative substitution replaces an amino acid with another amino acid having similar properties. Table 1 provides a list of groups of amino acids, where one member of the group is a conservative substitution for another member.

Table 1 : Conservative Substitutions

Ala, Val, Ile, Leu, Met
Ser, Thr,
Tyr, Trp
Asn, Gln
Asp, Glu
Lys, Arg, His

In additional embodiments the V_h region is either SEQ ID NO: 20, a humanized SEQ ID NO: 20 , or a de-immunized SEQ ID NO: 20; and/or the V_l region is either SEQ ID NO: 21, a humanized SEQ ID NO: 21, or a de-immunized SEQ ID NO: 21.

In different embodiments focusing on an antibody, the antibody comprises, consists, or consists essentially of: (a) a heavy chain comprising a V_h region as described in this Section III, and a human hinge, CH_1, CH_2, and CH_3 regions from an IgG_1, IgG_2, IgG_3 or IgG_4, and (b) a light chain comprising a V_l region as described above in this section III, and a human kappa C_L or human lambda C_L. In further embodiments: the antibody comprises, consists, or consists essentially of: (a) a heavy chain comprising a V_h region as described in this Section III, and a human hinge, CH_1, CH_2, and CH_3 regions from an IgG_1 or IgG_2 and (b) a light chain comprising a V_l region as described above in this Section III, and a human kappa C_L; and the heavy chain consists essentially of the amino acid sequence of SEQ ID NO: 22 and/or the light chain consists essentially of the amino acid sequence of SEQ ID NO: 23.

In additional embodiments the antigen-binding protein described herein has V_h and V_l regions providing an affinity K_D at least about 100 nM, preferably at least about 30 nM to the target antigen. Binding to the target antigen can be determined as described in Example 11, using an ORF0657n fragment from amino acids 42-486

Preferred binding proteins for the different embodiments are an antibody. More preferably the antibody is isolated or a monoclonal antibody.

IV. Protein Production

Antigen binding protein are preferably produced using recombinant nucleic acid techniques or through the use of a hybridoma. Recombinant nucleic acid techniques involve constructing

a nucleic acid template for protein synthesis. Hybridoma techniques involve using an immortalized cell line to produce the antigen binding protein. Suitable recombinant nucleic acid and hybridoma techniques are well known in the art. (See for example, Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 2005, Harlow *et al.*, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988.)

Recombinant nucleic acid encoding an antigen binding protein can be expressed in a host cell that in effect serves as a factory for the encoded protein. The recombinant nucleic acid can provide a recombinant gene encoding the antigen binding protein that exists autonomously from a host cell genome or as part of the host cell genome.

A recombinant gene contains nucleic acid encoding a protein along with regulatory elements for protein expression. Generally, the regulatory elements that are present in a recombinant gene include a transcriptional promoter, a ribosome binding site, a terminator, and an optionally present operator. A preferred element for processing in eukaryotic cells is a polyadenylation signal. Antibody associated introns may also be present. Examples of expression cassettes for antibody or antibody fragment production are well known in art. (*E.g.*, Persic *et al.*, *Gene 187*:9-18, 1997, Boel *et al.*, *J. Immunol. Methods 239*:153-166, 2000, Liang *et al.*, *J. Immunol. Methods* 247:119-130, 2001, Tsurushita *et al.*, *Methods 36*:69-83, 2005.)

Due to the degeneracy of the genetic code, a large number of different encoding nucleic acid sequences can be used to code for a particular protein. The degeneracy of the genetic code arises because almost all amino acids are encoded by different combinations of nucleotide triplets or "codons". Amino acids are encoded by codons as follows:

A=Ala=Alanine: codons GCA, GCC, GCG, GCU

C=Cys=Cysteine: codons UGC, UGU

D=Asp=Aspartic acid: codons GAC, GAU

E=Glu=Glutamic acid: codons GAA, GAG

F=Phe=Phenylalanine: codons UUC, UUU

G=Gly=Glycine: codons GGA, GGC, GGG, GGU

H=His=Histidine: codons CAC, CAU

I=Ile=Isoleucine: codons AUA, AUC, AUU

K=Lys=Lysine: codons AAA, AAG

L=Leu=Leucine: codons UUA, UUG, CUA, CUC, CUG, CUU

M=Met=Methionine: codon AUG

N=Asn=Asparagine: codons AAC, AAU

P=Pro=Proline: codons CCA, CCC, CCG, CCU

Q=Gln=Glutamine: codons CAA, CAG

R=Arg=Arginine: codons AGA, AGG, CGA, CGC, CGG, CGU

S=Ser=Serine: codons AGC, AGU, UCA, UCC, UCG, UCU

T=Thr=Threonine: codons ACA, ACC, ACG, ACU

V=Val=Valine: codons GUA, GUC, GUG, GUU

W=Trp=Tryptophan: codon UGG

Y=Tyr=Tyrosine: codons UAC, UAU

Expression of a recombinant gene in a cell is facilitated using an expression vector. Preferably, the expression vector, in addition to a recombinant gene, also contains an origin of replication for autonomous replication in a host cell, a selectable marker, a limited number of useful restriction enzyme sites, and a potential for high copy number. Examples of expression vectors for antibody and antibody fragment production are well known in art. (*E.g.*, Persic *et al., Gene 187*:9-18, 1997, Boel *et al., J. Immunol. Methods 239*:153-166, 2000, Liang *et al., J. Immunol. Methods* 247:119-130, 2001, Tsurushita *et al., Methods 36*:69-83, 2005.)

If desired, nucleic acid encoding an antibody may be integrated into the host chromosome using techniques well known in the art. (*E.g.*, Ausubel, *Current Protocols in Molecular Biology,* John Wiley, 2005, Marks *et al.,* International Application Number WO 95/17516, International Publication Date June 29, 1995.)

A variety of different cell lines can be used for recombinant antigen binding protein expression, including those from prokaryotic organisms (*e.g., E. coli, Bacillus sp,* and *Streptomyces sp.* (or streptomycete) and from eukaryotic (*e.g.,* yeast, Baculovirus, and mammalian). (Breitling *et al.,* Recombinant Antibodies, John Wiley & Sons, Inc. and Spektrum Akademischer Verlag, 1999, Kipriyanov *et al., Molecular Biotechnology 26*:39-60, 2004, Tsurushita *et al., Methods 36*:69-83, 2005.)

Preferred hosts for recombinant antigen binding protein expression provide for mammalian post translational modifications. Post translational modifications chemical modification such as glycosylation and disulfide bond formation. Another type of post translational modification is signal peptide cleavage.

Proper glycosylation can be important for antibody function. (Yoo *et al., Journal of Immunological Methods 261*:1-20, 2002, Li *et al., Nature Biotechnology 24*(2):210-215, 2006.) Naturally occurring antibodies contain at least one N-linked carbohydrate attached to a heavy chain. (Yoo *et al., Journal of Immunological Methods 261*:1-20, 2002.) Additional N-linked carbohydrates and O-linked carbohydrates may be present and may be important for antibody function. (*Id.*)

Different types of host cells can be used to provide for efficient post-translational modifications including mammalian host cells and non-mammalian cells. Examples of mammalian host cells include but are not limited to Chinese hamster ovary (Cho), HeLa, C6, PC12, Human Embryonic Kidney (HEK293) and myeloma cells. (Yoo *et al., Journal of Immunological Methods 261*:1-20, 2002, Persic *et al., Gene 187*:9-18, 1997.) Non-mammalian cells can be modified to replicate human glycosylation. (Li *et al., Nature Biotechnology 24*(2):210-215, 2006.) Glycoenginnered *Pichia pastoris* is an example of such a modified non-mammalian cell. (Li *et al., Nature Biotechnology 24*(2):210-215, 2006.)

Preferred recombinant genes comprise a nucleotide sequence encoding an antibody variable region that binds to a target region selected from the group consisting of: mAb IG3.BD4 target region, mAb 2H2.BE11 target region, mAb 13C7.BC1, and mAb 13G11.BF3 target region. A particular recombinant gene can encode for a protein containing one variable region or both a V_h and V_l region. The recombinant gene can also encode for antibody constant regions and hinge region. If desired, an antibody can be produced using a combination of recombinant genes, where one gene encodes for a light chain and the second gene encodes for a heavy chain.

Different embodiments are provided by the nucleic acid encoding a protein described in Section II or III *supra*. Examples of such embodiments are provided below.

In an embodiment directed to a V_h encoding region, the nucleotide sequence encodes a variable region comprising, consisting, or consisting essentially of: a first V_h CDR comprising, consisting, or consisting essentially of amino acids 36-45 of SEQ ID NO: 20 or a sequence differing from amino acids 36-45 by one amino acid; a second V_h CDR comprising, consisting, or consisting essentially of amino acids 50-65 of SEQ ID NO: 20 or a sequence differing from amino acids 50-65 by one amino acid; and a third V_h CDR comprising, consisting, or consisting essentially of amino acids 98-107 of SEQ ID NO: 20 or a sequence differing from amino acids 98-107 by one amino acid.

In an embodiment directed to a V_l encoding region, the nucleotide sequence encodes a variable region comprising, consisting, or consisting essentially of a first V_l CDR comprising, consisting, or consisting essentially of amino acids 24-33 of SEQ ID NO: 21 or a sequence differing from amino acids 24-33 by one amino acid; a second V_l CDR comprising, consisting, or consisting essentially of amino acids 49-55 of SEQ ID NO: 21 or a sequence differing from amino acids 49-55 by one amino acid; and a third V_l CDR comprising, consisting, or consisting essentially of amino acids 88-96 of SEQ ID NO: 21 or a sequence differing from amino acids 88-96 by one amino acid.

In additional embodiments: the V_h region is either SEQ ID NO: 20, a humanized SEQ ID NO: 20 , or a de-immunized SEQ ID NO: 20; and the V_l region is either SEQ ID NO: 21, a humanized SEQ ID NO: 21, or a de-immunized SEQ ID NO: 21.

In different embodiments focusing on an antibody heavy and/or light chain, the recombinant gene encodes either or both a protein comprising, consisting, or consisting essentially of: (a) a heavy chain comprising a V_h region as provided in Section III *supra,* a human hinge, CH_1, CH_2, and CH_3 from an IgG1, IgG2, IgG3 or IgG4 subtype or (b) a light chain comprising a V_l region as provided in Section III *supra,* and a human kappa C_L or lambda C_L. In a further embodiment the heavy chain consists essentially of the amino acid sequence of SEQ ID NO: 22; and the light chain consists essentially of the amino acid sequence of SEQ ID NO: 23.

V. Applications of Antigen Binding Proteins.

Antigens containing certain ORF0657n regions can be used to provide a protective immune response against *S. aureus* infection. (Anderson *et al.*, International Publication No. WO

2005/009379, International Publication Date February 3, 2005.) An antigen binding protein recognizing an ORF0657n target region can be used to facilitate the production, characterization, or study of ORF0657n antigens and vaccines. Antigen binding protein recognizing appropriate epitopes can also have therapeutic applications.

Examples of different uses in the production, characterization, or study of ORF0657n related antigens and vaccines include:

1) Identifying the presence of an ORF0657n antigen, for example, by Western blot;
2) Identifying the presence of an ORF0657n antigen on a cell surface, for example, by flow cytometry. This is useful, for example, in determining expression on multiple strains of *S. aureus* as well as confirmation of knock-out mutants;
3) Passive protection experiments. The antibodies can be used in a lethal model to determine if a specific area of the ORF0657n protein confers protection;
4) An immunoassay. The assay can be used to monitor antigen quality, product production and stability;
5) As a control in mouse potency assays to monitor immunogenicity of an antigen vaccine product; and
6) Serology assays can utilize a monoclonal antibody in a competitive format to identify an immune response to ORF0657n derived antigen vaccinated patients.

Techniques for using antigen binding proteins, such as monoclonal antibodies, in the production, characterization, or study of a target protein are well known in the art. (See, for example, Ausubel, *Current Protocols in Molecular Biology,* John Wiley, 2005, Harlow *et al., Antibodies, A Laboratory Manual,* Cold Spring Harbor Laboratory, 1988, Harlow *et al., Using Antibodies,* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory Press, 1999, Lipman *et al., ILAR Journal 46*:258-268, 2005.)

In an embodiment of the present invention, the presence of an ORF0657n antigen in a solution, bound to a microsphere or on a cell is determined using an antigen binding protein. The ability of the binding protein to bind to a protein present in the solution or cell can be determined using different techniques such as a Western blot, enzyme-linked immunosorbent assay (ELISA), flow cytometry, and Luminex immunoassay.

VI. Treatment

Therapeutic and prophylactic treatment can be performed on a patient using an antigen binding protein binding to an appropriate target region. Therapeutic treatment is performed on those persons infected with *S. aureus.* Prophylactic treatment can be performed on the general population or a subset of the general population. A preferred subset of the general population are those persons at an increased risk of *S. aureus* infection.

A "patient" refers to a mammal capable of being infected with *S. aureus*. Preferably, the patient is a human. However, other types of mammals such as cows, pigs, sheep, goats, rabbits, horses, dogs, cats, monkeys, rats, and mice, can be infected with *S. aureus*. Treatment of non-human patients is useful in protecting pets and livestock, and in evaluating the efficacy of a particular treatment.

Persons with an increased risk of *S. aureus* infection include health care workers; hospital patients; patients with a weakened immune system; patients undergoing surgery; patients receiving foreign body implants, such as a catheter or a vascular device; patients facing therapy leading to a weakened immunity; and persons in professions having an increased risk of burn or wound injury. (*The Staphylococci in Human Disease*, Crossley and Archer (ed.), Churchill Livingstone Inc. 1997.)

In an embodiment, a patient is administered an antigen binding protein in conjunction with surgery or a foreign body implant. Reference to "surgery or a foreign body implant" includes surgery with or without providing a foreign implant, and providing a foreign implant with or without surgery. The timing of administration can be designed to achieve prophylactic treatment and/or therapeutic treatment. Administration is preferably started around the same time as surgery or implantation.

Guidelines for pharmaceutical administration in general are provided in, for example, *Remington's Pharmaceutical Sciences 20th Edition*, Ed. Gennaro, Mack Publishing, 2000; and *Modern Pharmaceutics 2nd Edition*, Eds. Banker and Rhodes, Marcel Dekker, Inc., 1990.

Pharmaceutically acceptable carriers facilitate storage or administration of an antigen binding protein. Substances used to stabilize protein solution formulations include carbohydrates, amino acids, and buffering salts. (Middaugh *et al.*, Handbook of Experimental Pharmacology *137*:33-58, 1999.)

Antigen binding proteins can be administered by different routes such as intraveneous, subcutaneous, intramuscular, or mucosal. Subcutaneous and intramuscular administration can be performed using, for example, needles or jet-injectors. Mucosal delivery, such as nasal delivery, can involve using enhancers or mucoadhesives to produce a longer retention time at adsorption sites. (Middaugh *et al.*, *Handbook of Experimental Pharmacology 137*:33-58, 1999.)

Suitable dosing regimens are preferably determined taking into account factors well known in the art including age, weight, sex and medical condition of the patient; the route of administration; the desired effect; and the particular compound employed. It is expected that an effective dose range should be about 0.1 mg/kg to 20 mg/kg, or 0.5 mg/kg to 5 mg/kg. The dosing frequency can vary depending upon the effectiveness and stability of the compound. Examples of dosing frequencies include biweekly, weekly, monthly and bimonthly.

VII. Examples

Examples are provided below further illustrating different features of the present invention. The examples also illustrate useful methodology for practicing the invention. These examples do not limit the claimed invention.

Example 1: Generation of Monoclonal Antibodies to ORF0657n

Monoclonal antibodies directed to ORF0657n (SEQ ID NO: 1) were generated using ORF0657n-C/e (SEQ ID NO: 2) or ORF0657n-H/y (SEQ ID NO: 3) as an antigen. The antibodies were identified and characterized by ELISA and flow cytometry.

Mice and Immunizations: Female BALB/c mice, 4-5 weeks old, were purchased from Taconic (Germantown, N. Y.). Mice were immunized intramuscularly (i.m.) on days 0, 7, and 21, with 20 μg of *E. coli* produced ORF0657n-C/e antigen or Yeast expressed ORF0657n-H/y antigen, formulated on aluminum hydroxyphosphate adjuvant. (Anderson *et al.*, International Publication No. WO 2005/009379, International Publication Date February 3, 2005.) A final intravenous injection (i.v.) of 20 μg of protein in phosphate buffered saline (PBS) was given to mice three days prior to the fusion. Mice were sacrificed and the spleens removed for cell fusion.

MAb Production: Lymphocytes prepared from spleens were fused with the mouse myeloma partner SP2/0-Ag14 (ATCC 1581) by polyethylene glycol 1500 (Boehringer Mannheim) at a ratio of 3:1. The fusions were plated into 96-well flat-bottomed microtiter plates in Dulbecco's Modification of Eagle's Medium, high glucose, pyruvate (DMEM) containing 20% fetal bovine serum, hypoxanthine (10^{-4} M), thymidine (10^{-5} M), Aminopterin (4×10^{-7} M) was added 24 hours later. Supernatants from growing hybridomas were screened by ELISA for reactivity to ORF0657n as described below. Positive wells were cloned by limiting dilution and retested for ELISA reactivity. Monoclonal antibodies were classified with an antibody-isotyping kit (Roche Diagnostics Corporation, Indianapolis, IN).

ELISA: Costar medium binding microtiter plates were coated overnight at 2-8°C with 50 nanograms per well of *E. coli* expressed SEQ ID NO: 2 in PBS. The plate was washed three times with PBS, 0.05% Tween20 and blocked with 1% Bovine serum albumin, PBS, 0.05% Tween20 (assay diluent) for at least 1 hour. The plate was washed as before and supernatants from the fusion wells or cloned hybridomas were added and allowed to incubate for 2 hours at room temperature. The plate was washed as before and a Goat anti-mouse IgG (H+L)-HRP conjugate (Zymed) (1:8000 in assay diluent) added and allowed to incubate for 1 hour at room temperature. Assay plates were developed with TMB substrate, the reaction stopped with 2.0 N H_2SO_4 and read in a plate reader at OD 450 nm. Wells were considered positive that had an optical density at 450 nm of >1.0.

Flow Cytometry: Prepared glycerol stocks of *S. aureus* passaged under iron-starved conditions (in RPMI) were used to evaluate mAb for ORF0657n binding. Frozen glycerol stock cells were thawed and resuspended in PBS; 1% bovine serum albumin; 0.1% sodium azide, 0.2% Pig IgG (Sigma) (PAAG) to a concentration of 5×10^7 CFU/50 ul. A 50 ul aliquot of the cells were placed in a 1.5 ml Eppendorf tube per reaction. Fifty microliters of the hybridoma culture were added to each reaction tube and incubated for 1 hour at room temperature. The cells were washed by adding 1 mL of phosphate buffered saline; 1% bovine serum albumin; 0.1% sodium azide (PAA) to the tube. The cells

were pelleted by centrifugation (5500 rpm, 5 minutes). The supernatant was removed and the cells were mixed with 100 µL of secondary antibody (FITC-labeled goat anti-mouse Ig (BD Pharmingen) diluted 1:100 in PAAG). Incubation was for 1 hour at room temperature in the dark. After incubation, 1 mL PAA was added to the reaction mixture, the cells were pelleted (5500 rpm, 5 minutes) and supernatant removed. The pellets were resuspended in 1 mL of PBS and transferred to 12 x 75 mm tubes for FAC analysis.

Tubes were run on a BD-FACSCalibur flow cytometer instrument gated for bacterial cells and measuring the amount of FITC associated with the cells. A standard antibody with known binding to the surface of *S. aureus* was run in every assay. A negative control was run as cells and the secondary conjugate alone. Hybridoma wells were considered positive if the geometric mean value was greater than 30.

Two separate fusions resulted in a panel of twelve monoclonal antibodies (mAb). All of the mAbs were reactive in ELISA (Table 2). Ten of the twelve mAbs bound to the surface of bacteria as evidenced by flow cytometry. All of the mAbs were positive by Western Blot analysis with the wild type protein.

Table 2

mAbs/cell lines Fusion #1	mAbs/cell lines Fusion #2
1) 2H2.B8 IgG1	
2) 8H6.E11.H3 IgG2a*	
3) 7H2.C11 IgG1*	
	4) 2E12.A8 IgG1
	5) 8A8.B4 IgG1
	6) 3G11.D5 IgG1
	7) 13G11.C11 IgG1
	8) 13C7.D12 IgG1
	9) 1G3.B3 IgG1
	10) 9H3.E4 IgG1
	11) 3B7.G8 IgG1
	12) 3G12.A4 IgG1

* Not reactive in flow cytometry. Fusion #1 was generated from *E. coli* produced ORF0657n-C/e antigen. Fusion #2 was generated with Yeast expressed ORF0657n-H/y antigen.

Example 2: Class Switching mAbs

All of the mAbs isolated that bound to the native antigen were of the IgG1 isotype. These antibodies were class switched to an IgG2b isotype by selecting for shift variants (Spira *et al, J. of Immunogical Methods, 74*:307-315, 1985). A suitable immunoassay was developed using an IgG2b conjugate and the cell line was plated at a high density. Somatic cell mutations were selected, enriched

and then cloned. The binding site of the switched mAb remained identical to the original mAb, but switching to an IgG2b subtype gave a more favorable isotype (initiating the complement cascade) in the passive protection studies.

Table 3 Class Switched mAbs

IgG1 isotype	IgG2b isotype
2H2.B8	2H2.BE11
2E12.A8	2E12.BG1
8A8.B4	8A8.BF9
3G11.D5	3G11.BE5
13G11.C11	13G11.BF3
13C7.D12	13C7.BC1
1G3.B3	1G3.BD4
9H3.E4	9H3.BE4

Example 3: Binding Inhibition Studies with Native Antigen

Purified antibodies were labeled with Alexafluor-488 using a mAb labeling kit (Molecular Probes) according to the manufacturer's instructions. The amount of mAb that would just saturate the surface of RPMI-grown bacterial cells was determined for both the labeled and unlabeled mAbs. Each of the mAbs in Table 3 (1st column) were used labeled and unlabeled.

The inhibition assay was performed by first incubating 5 X 10^7 cells with the unlabeled mAb at a concentration that would saturate the surface of the cells. This reaction was incubated at room temperature for 1 hour. After this incubation, the reactions were washed with 1 ml of PAA and spun at 6,000 RPM for 5 minutes in a microcentrifuge (Hermle). The supernatant was removed down to ~50 ul and the cells were resuspended in 100 ul of PAAG containing the amount of directly labeled mAb that would just saturate the surface of the cells. After this incubation, the reactions were washed with 1 ml of PAA and spun at 6,000 RPM for 5 minutes in a microcentrifuge (Hermle). The supernatant was removed down to ~50 ul and the cells were resuspended in 1 ml of PBS and transferred to 12 x 75 mm tubes for FAC analysis. As controls, separate reactions with the unlabeled mAb were measured with a secondary Alexafluor-488 conjugated goat anti-mouse IgG (H+L) (Molecular probes, 1:400 in PAAG) to determine that this mAb was bound to the surface. A positive control was also performed that had only the labeled mAb with the cells. If the unlabeled mAb bound to the same epitope as the labeled mAb then there would be no or low fluorescent reactivity associated with the cells. If the unlabeled mAb bound to a different epitope than the labeled mAb then the level of reactivity associated with the surface would be equivalent to the labeled mAb only control cells.

The panel of monoclonal antibodies fell into four reactive groups by inhibition studies:

Table 4

Group I	Group II	Group III	Group IV
2H2.B8	9H3.E4	13G11.C11	2E12.A8
8A8.B4	1G3.B3		13C7.D12
	3G11.D5		

Example 4: Binding Studies with Denatured Antigen and Altered Antigens

ORF0657n altered proteins were used to further characterize binding. Nucleic acid encoding ORF0657n was initially cloned into the expression vector pET-28a (Novagen) and expressed in *E. coli* with a C-terminal 6X his tag (SEQ ID NO: 2). The expression vector with the cloned gene was subjected to mutagenesis using Stratagene's QuikChange XL Site-Directed Mutagenesis Kit following the manufacturer's instructions. The gene was mutated with specific sequential amino acid changes. The resulting plasmid was transformed into Stratagene's XL10-Gold competent cells following the manufacturer's protocol. Plasmids were isolated from transformants using Qiagen's QIAprep Spin Miniprep Kit. Transformants were screened by sequencing using ABI's 310 DNA Sequencer. Plasmid from the transformant exhibiting the greatest number of base changes was transformed into the expression host HMS174(DE3) (Novagen). Transformants were expressed following Novagen's instructions.

Different ORF0657n altered proteins were used to determine the diversity of the ORF0657n mAbs (SEQ IDs 4-19). These proteins were screened with the 10 different mAbs in dot blots using standard procedures. Positive/negatives were confirmed by Western blots using standard procedures. By this approach antibodies were grouped according to their binding profile. Seven of the antibodies resolved to three groups; the three remaining antibodies (2H2.B8, 8A8.E11.H3 and 13G11.C11) had profiles that were similar but not identical to each other (Table 5).

TABLE 5: Binding of ORF0657n specific mAbs to ORF0657n mutant proteins detected by Western blot

	Group III		Group II			Group IV		Group I		
SEQ ID NO:	3G11.C11	3G12.A4	3B7.G8	1G3.B3	9H3.E4	2E12.A8	13C7.D12	2H2.B8	8A8.E11.H3	13G11.C11
1	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+

TABLE 5: Binding of ORF0657n specific mAbs to ORF0657n mutant proteins detected by Western blot

	Group III		Group II			Group IV		Group I		
SEQ ID NO:	3G11.C11	3G12.A4	3B7.G8	1G3.B3	9H3.E4	2E12.A8	13C7.D12	2H2.B8	8A8.E11.H3	13G11.C11
4	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	-	-	+	+	+
7	+	+	+	+	+	-	-	+	+	+
8	+	+	+	+	+	-	-	+	+	+
9	+	+	+	+	+	-	-	-	+	+
10	+	+	+	+	+	-	-	-	+	+
11	-	-	W	W	W	-	-	-	-	W
12	-	-	W	W	W	-	-	-	-	W
13	-	-	-	-	-	-	-	-	-	-
14	+	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	-	+	+
17	+	+	+	+	+	+	+	-	+	+
18	+	+	+	+	+	+	+	-	+	-
19	+	+	+	+	+	W	W	-	-	+

+, Antibody bound to protein in a Western; -, Antibody did not bind to protein by Western; W, Weak binding of antibody to protein detected by Western. Antibodies were grouped according to hybridization profile. A dotted line is used where similar, but not identical profiles were obtained.

Example 5: BAIcore Studies

In BIAcore studies the mAbs were examined by "footprint analysis" using purified ORF0657n-H/y as the antigen. Pair-wise binding experiments were conducted using real-time biomolecular interaction analysis via BIACORE ®. BIACORE ® incorporates microfluidics technology and surface plasmon resonance (SPR) to detect changes in mass by monitoring changes in the refractive index of a polarized light aimed directly at the surface of a carboxyl methyl dextran coated (CM5) sensor chip. The changes in response, measured in Response Units, can be correlated to the amount of bound analyte (*i.e.* antigen or antibody).

An anti-staphylococcal antibody (mAb 13C7.D12) was covalently bound (immobilized) on the surface of the CM5 sensor chip. The immobilized Ab was exposed first to the ORF0657n protein and subsequently to a pair of antibodies in a matrix format. After each cycle of ORF0657n protein + antibody pair, the surface of the sensor chip was regenerated back to the immobilized mAb 13C7.D12

using 20 mM HCl. Eight antibodies were tested against the ORF0657n protein in a matrix format so that all combinations of each antibody pair could be analyzed. The matrix design for mAb pairs used in this experiment is summarized in Table 6.

Table 6. Summary of Antibodies Tested in 8x8 Matrix

		Second Antibody			
Cycle #	First Antibody	Flow Cell 1	Flow Cell 2	Flow Cell 3	Flow Cell 4
1	N/A Immobilization	13C7.D12	13C7.D12	13C7.D12	13C7.D12
2	2H2.B8	2H2.B8	13C7.D12	8A8.B4	9H3.E4
3	2H2.B8	13G11.C11	2E12.A8	1G3.B3	3G11.D5
4	13C7.D12	2H.B82	13C7.D12	8A8.B4	9H3.E4
5	13C7.D12	13G11.C11	2E12.A8	1G3.B3	3G11.D5
6	8A8.B4	2H2.B8	13C7.D12	8A8.B4	9H3.E4
7	8A8.B4	13G11.C11	2E12.A8	1G3.B3	3G11.D5
8	9H3.E4	2H2.B8	13C7.D12	8A8.B4	9H3.E4
9	9H3.E4	13G11.C11	2E12.A8	1G3.B3	3G11.D5
10	13G11.C11	2H2.B8	13C7.D12	8A8.B4	9H3.E4
11	13G11.C11	13G11.C11	2E12.A8	1G3.B3	3G11.D5
12	2E12.A8	2H2.B8	13C7.D12	8A8.B4	9H3.E4
13	2E12.A8	13G11.C11	2E12.A8	1G3B3	3G11.D5
14	1G3.B3	2H2.B8	13C7.D12	8A8.B4	9H3.E4
15	1G3.B3	13G11.C11	2E12.A8	1G3.B3	3G11.D5
16	3G11.D5	2H2.B8	13C7.D12	8A8.B4	9H3.E4
17	3G11.D5	13G11.C11	2E12.A8	1G3.B3	3G11.D5

To normalize for the amount of antigen initially bound (captured) in each run, the following ratio for each test antibody/antigen complex is calculated:

$$= \frac{\text{Test Antibody Response Units* 1000}}{\text{ORF0657n protein Response Units}} \quad \text{or} \quad \frac{mRU_{Ab}}{RU_{Ag}}$$

The percentage of available epitope remaining for each antibody can be calculated for the mapping pair as follows:

$$= \frac{(mRU_{Ab} \text{ (when } 2^{nd} \text{ Ab)} / RU_{Ag})*100}{(mRU_{Ab} \text{ (when } 1^{st} \text{ Ab)} / RU_{Ag})} \quad \text{or} \quad \text{\% Remaining (calculated for each Ab)}$$

Figure 2 illustrates matrix resulting outlining the reactivities of the monoclonal antibodies in a pair-wise binding study. The panel of monoclonal antibodies fell into three reactive areas by the BIACORE® method (See Table 7).

Table 7

Group I	Group II	Group III
2H2.B8	13G11.C11	13C7.D12
8A8.B4	3G11.D5	2E12.A8
9H3.E4		
1G3.B3		

Example 6: Protection Studies with Passive Immunization in a Murine Sepsis Model

The monoclonal antibodies mAb 2H2.BE11 and mAb 13C7.BC1 were tested for their ability to provide protection against *S. aureus* infection. These antibodies recognize different epitopes on the ORF0657n protein. Controls included an isotype matched mAb and PBS-only.

The mAbs or PBS were administered intraperitoneally (i.p.) 20 hours prior to bacterial challenge. Mice were then challenged with a LD_{80-90} dose of *S. aureus* Becker i.v. and monitored for survival. Each experiment was repeated three times with groups of 10 or 20 mice and was monitored for 10 days. The half life for the monoclonal antibodies in uninfected BALB/c mice is approximately eight days. A dose of 0.5 mg was found to be optimal. The results of experiments with the two monoclonal antibodies are presented in Figures 3A-C, 4A, 4B, and 5A-C.

Whereas the mAb 13C7.BC1 significantly improved survival at day 10 compared to the controls in one experiment, in the other 2 repetitions the overall survival rate was similar to that of the controls (Figures 3A-3C). However, compared to controls, there was delay in the time to death of the mAb 13C7.BC1 treated mice within this 10 day period. A similar trend in delay of time to death of the mAb 2H2.BE11 treated mice was also noted in two of the three experiments (Figures 5A-5C).

The effect of mAb 13C7.BC1 was also examined using a recent *S. aureus* clinical isolate UK58 (Figures 4A and 4B). This strain was minimally passaged from an abscess site in a patient. In two independent experiments, the results show a delay in time to death with the UK58 challenge.

Antibody persistence studies cannot be evaluated in the LD_{80-90} model due to the rapid rate of death. Therefore, a sub-lethal challenge model was run. In the sub-lethal model the challenge dose used is 10% of that used for the LD_{80-90} model. The sub-lethal challenge model was monitored over a four day period. Groups of 22 mice received 0.5 mg doses of either mAb 13C7.BC1 or isotype control mAb (6G6) 20 hours prior to i.v. bacterial challenge with 5×10^7 CFU of *S. aureus* Becker. Two animals from each group were sacrificed just prior to challenge (T=0) to determine the mAb levels in the

serum at the time of challenge. At 2, 24, 48, 72 and 96 hours post challenge, four mice from each group were sacrificed and serum mAb levels determined.

From this sub-lethal challenge experiment, the half life of mAb 13C7.BC1 in *S. aureus*-infected mice was estimated to be approximately one-day. In contrast, the half life of the isotype control mAb was estimated to be greater than four days (data not shown). These data point to a specific reduction of mAb 13C7.BC1 in *S. aureus* challenged mice, which appears to be exhausted well before the ten day period monitored in the lethal model.

In six of the eight experiments illustrated in Figures 3A-C, 4A, 4B, and 5A-C, improved survival was observed through approximately three days for the groups receiving the mAb administration. These results provide an indication that such mAbs have a positive effect on the survival rate of *S. aureus* challenged mice.

Example 7: Protection Studies with Passive Immunization in a Murine Indwelling Catheter Model

A murine indwelling catheter model was used with mAb 2H2.BE11. The *S. aureus* strain used in this model was the clinical isolate MCL8538. This strain was selected as lower inocula could be administered while still getting reproducible colonization of catheters compared to *S. aureus* Becker, the strain used in the murine sepsis model.

ICR mice had catheters (PE50 silicone rubber) surgically implanted into the jugular vein, held in place with sutures, and exiting with a port on the dorsal midline of the mouse. Mice were rested 9-11 days post surgery. At 24 hours prior to challenge, mice were passively immunized with a single injection of 600 mcg of murine monoclonal antibody 2H2.BE11 administered i.p. At day 0, mice were challenged with *S. aureus* MCL8538 administered i.v. The inoculum dose was 2 -8 X 10^5 CFU in 100 µl volume (Experiments 1 to 3). This low dose was found to clear spontaneously from the catheters after 4 days. Therefore, catheters were assessed for bacteria at 24 hours post challenge. At that time, mice were sacrificed and catheters harvested. The presence of bacteria on the catheters was assessed by culturing the entire catheter on TSA. If any sign of outgrowth was observed on the plate the catheter was scored as culture positive.

In two of the first three experiments, the number of culture negative catheters was significantly lower in mice passively immunized with antibody 2H2.BE11, as compared to the isotype control antibody. A fourth experiment was performed using a larger inoculum dose. In this more rigorous challenge, the dose was determined to be one in which 100% of catheters were reproducibly infected, and this infection was not spontaneously cleared by control mice (monitored over 7 days). In experiment 4, with the larger inoculum size, again, significantly fewer catheters were found to be infected in mice injected with antibody to 2H2.BE11, compared with the isotype control. Results of the four experiments are summarized in Table 8.

Table 8: Number Of Culture Negative Catheters Obtained In 4 Independent Passive Transfer Experiments Using a Murine Indwelling Catheter Model

	Number of Culture-Negative Catheters					p-value
Monoclonal	Exp#1	Exp#2	Exp#3	Exp#4	Total	
2H2.BE11	3 of 4 (75%)	6 of 8 (75%)	4 of 10 (40%)	4 of 9 (44%)	17/31 (54%)	0.0187
Isotype matched control	1 of 4 (25%)	3 of 8 (38%)	4 of 10 (40%)	0 of 9 (0%)	8/31 (25%)	

Groups of ICR mice with indwelling catheters were injected i.p. with 600 mcg of murine monoclonal antibody 24 hours prior to challenge, all monoclonals of the IgG2b isotype

Example 8: Ex-Vivo Pre-Opsonization of Bacteria Using anti-ORF0657n Monoclonal Antibodies 2H2.B8 (IgG1), 2H2.BE11 (IgG2b), or 13C7.IgG2b or Isotype Matched Control mAbs

To test whether monoclonal antibodies to ORF0657n are opsonic, passive protection experiments were conducted in which a lethal dose of *S. aureus* was pre-opsonized with the monoclonal antibodies 2H2.B8, 2H2.BE11, or 13C7.IgG2b, or an isotype matched control monoclonal antibody. Pre-opsonized bacteria were then administered to mice i.p. Bacteria used in these experiments were *S. aureus* RN4220 (wild type) or RN4220.0657n. The RN4220.0657n bacteria were engineered to express ORF0657n in the absence of control by the FUR box. Therefore, they could be grown in the presence of iron and still express ORF0657n antigen on their surface. Alternatively, RN4220 (wild type) was passed 2X in a low iron medium RPMI to induce expression of 0657n on the bacteria surface.

A quantity of bacteria sufficient for 6 Balb/c mice (6 X LD_{100}) was incubated with 800 µg IgG at 4 °C for 1 hour, with gentle rocking. Bacteria were then pelleted and any unbound mAb removed. Antibody-opsonized bacteria were re-suspended in 2.4 mL of PBS, and 0.4 mL (1 X LD_{100}) was injected into each of five mice. After challenge, each inoculum was quantitated by plating on TSA to insure that equivalent CFU was given to all groups of mice and that the mAbs had not aggregated the bacteria. Survival was monitored for 3 days post challenge. Since the target antigen must be present on the surface of the bacteria for this procedure to be effective, care was taken to ensure that 0657n was expressed on the bacteria prior to opsonization. ORF0657n expression was monitored by flow cytometry using mAb 2H2.B8. The dose of opsonized bacteria injected into each mouse was 2-4 X 10^9 CFU RN4220.0657n/mouse, or 1-2 X 10^9 CFU RN4220(2X RPMI)/mouse.

When pre-opsonized with either 2H2.B8 or 2H2.BE11, but not an isotype matched control mAb, mice were protected from death from a lethal dose of RN4220.0657n staphylococci. The experiment was repeated twice for the IgG1 isotype and three times for the IgG2b isotype with similar results (Table 9A).

Table 9A: Ex-vivo Protection with Anti-0657n mAb

Monoclonal	Exp 1 Surviving Mice	Exp 2 Surviving Mice	Exp 3 Surviving Mice	Total
2H2.BE11 (IgG2b)	5	4	5	93% (14/15)
6G6.A8 (IgG2b)	1	0	1	13% (2/15)
PBS	1	2	0	20% (3/15)
2H2.BE11 (IgG1)	ND	4	5	90% (9/10)
10B4.H4 (IgG1)	ND	1	1	20% (2/10)

Five mice were used in each experiment. Challenge strain RN4220.0657n.pYZ119. Dose: 2-4 X 10^9 CFU. Test mAbs: murine anti-0657n 2H2.BE11 (IgG2b); 2H2.B8 (IgG1).

When pre-opsonized with either mAb 2H2.B8 but not an isotype matched control mAb, mice were protected from death from a lethal dose of RN4220 (2X RPMI) staphylococci. The experiment was repeated six times with similar results (Table 9B).

Table 9B: Ex-vivo Protection with Anti-0657n mAb

Monoclonal	# Tests	Aggregate	% Survival
2H2.B8	6	30/30	100%
10B4.IgG1 Isotype control	6	2/30	7%
13C7.IgG2b	2	0/10	0%
6G6.IgG2b Isotype control	2	0/10	0%

Murine anti-0657n 2H2 was very effective in preventing death in this lethal model. The 13C7 mAb was not effective in this model (as opposed to the previously described model illustrated in Figures 3-6). All (2H2.BE11, 2H2.B8 and 13C7.IgG2b) of the anti-0657n mAb's bind RN4220 (as demonstrated using flow cytometery) and all have opsonizing activity in the *in vitro* OPA assay. This model reflects an additional requirement for epitope specificity for enhancing survival in the peritoneum of the mouse.

Example 8: Epitope mapping studies performed with 2H2 mAb

The experiments described in this example provide evidence that the monoclonal antibody 2H2.BE11 recognizes a conformational epitope within ORFO657n. The experiments localized the minimal sequence within ORFO657n required for displaying the conformational epitope in a three dimensional structure recognized by 2H2 mAb. In addition, the experiments identified distinct lysine

residues within the minimal sequence of ORFO657n that become protected from reacting with small molecules when 2H2 mAb is bound to ORFO657n.

The potential ability of 2H2 mAb to recognize linear epitopes of typically 9 to 14 amino acids in length within the sequence of ORFO657n was investigated using epitope extraction and starting with an ORF0657n fragment from amino acid 42 to amino acid 486 of SEQ ID NO: 1 ("ORF0657t"). In detail: 30 ug of 2H2 mAb were immobilized by chemical cross linking to 10 mg of cyanogen bromide activated sepharose (Amersham cat. No. 17 0430 01) for each of the epitope extraction experiments. Proteolytic digests of the ORF0657t were generated with GluC (Roche Applied Science cat. No. 11 420 3997 001), Asp-N (Roche Applied Science cat. No. 11 054 589 001) or Chymotrypsin (Roche Applied Science cat. No. 11 418 467 001) and characterized by 1D/LC-MS/MS on a linear ion trap (LTQ – Thermo Electron Inc). In three individual experiments 8.4 µg of the characterized proteolytic digest from any protease was allowed to react with the immobilized antibody. Unbound peptides were washed off the antibody cross-linked beads. Potentially bound peptides were eluted with low pH and characterized by 1D/LC-MS/MS. None of the generated proteolytic peptides were recognized with high efficiency and specificity by 2H2 mAb, providing a strong indication that 2H2 mAb did not recognize a linear epitope.

The finding that 2H2 mAb did not recognize a linear sequence of ORFO657n was corroborated by a limited chemical cleavage experiment. ORF0657t was chemically cleft with CNBr for 2 hours. The resulting cleavage products were analyzed by SDS-PAGE. SDS-PAGE analysis showed 5 major bands with molecular weights of approximately 42 kDa, 35 kDa, 25 kDa, 15 kDa and 10 kDa. A Western Blot analysis with 2H2 mAb clearly showed that only the 42 kDa band was recognized by 2H2. All bands were excised from the SDS-PAGE, in-gel digest was performed, and the resulting peptides that were identified by tandem mass spectrometry were matched to corresponding sequences in ORF0657t. The result of the analysis of the major bands is shown in Table 10:

Table 10

CNBr cleavage	Binds to 2H2 mAb	ORFO657t	Calculated MW kDa
Band 42 kDa	yes	[001-356]	40.7
Band 35 kDa	no	[001-323]	36.7
Band 25 kDa	no	[001-214]	23.9
		[116-302]	21.9
Band 15 kDa	no	[215-356]	16.8
		[303-446]	16.6
Band 10 kDa	no	[114-214]	11.7
		[215-302]	10.39
		[357-446]	10.28

The importance of a fragment with a molecular weight of ~ 42 kDa was confirmed by epitope excision. In detail, 210 µg of 2H2 mAb was immobilized by chemical cross linking to 50 mg of cyanogen bromide activated sepharose (Amersham cat. No. 17 0430 01) for each of the epitope excision experiments. Then, 50 µg of intact ORF0657t was allowed to bind to the immobilized antibody and non-bound ORF0657t washed off by intensive washing with phosphate buffered saline. In three independent experiments proteases Glu-C, Trypsin and a sequential combination of GluC, AspN, Trypsin, Chymotrypsin, and Carboxy-peptidase Y were added for 5 hours or one hour per protease in the sequential combination. Peptides that were excised by the proteases during the incubation were thoroughly washed away and ORF0657t fragments that specifically bound to 2H2 mAb released with SDS loading buffer.

Fragments that specifically bound to 2H2 mAb were analyzed by SDS-page. All three of the epitope excision experiments showed exclusively one band with a molecular weight between 40 and 42 kDa in the SDS-Page analysis. Bands binding to 2H2 mAb were confirmed by Western Blot analysis. The epitope excision experiment was repeated for the Glu-C protease. This time the fragment of ORF0657t that specifically bound to 2H2 mAb was released with acidic conditions and analyzed by 1D/LC-MS/MS on a linear ion trap (LTQ, Thermo Electron). The eluted sample showed a signal (total ion count) with the expected intensity at 82-87 minutes (40%– 45% acetonitrile) and multiple charge states ($[M+67\ H]^{67+}$ to $([M+30\ H]^{30+}$) that deconvoluted to 42.628 kDa. A possible fragment of ORFO657t corresponding to this particular mass is sequence [012-382] of ORFO657t with a molecular weight of 42.6 kDa.

To determine which lysine residues of ORFO657t are protected from chemical reactions upon binding of 2H2 mAb, chemical labeling experiments were preformed with sulfo-NHS-acetate (Pierce Cat. No. 26777) using three different experimental conditions in the presence or absence of 2H2 mAb. See Table 11.

Table 11

Experiment	1	2	3
molar excess 2H2 mAb	0 or 3	0 or 3	0 or 3
molar excess sulfo-NHS acetate	25	500	75
Reaction temperature °C	room temperature	15	37
Reaction time	1 hour	30 minutes	2 hours

For each experiment, reaction products produced with O or 3 molar excess 2H2 mAb were incubated with one of three proteases resulting in 2 x 9 reaction mixtures. Experiment 1 employed

GluC, AspN and Trypsin. Experiments 2 and 3 employed GluC, AspN, and Chymotrypsin. The proteolytic peptides were then analyzed by 1D/LC-MS/MS. For each of the reactions a ratio of acetylated and non-acetylated lysine residues was calculated based on the area under curve of the total ion count (TIC) of the individual peptides. Obtained ratios were then compared between the pairs (with and without 2H2 mAb) for identical reaction conditions. A global analysis was performed for all three reaction conditions to identify lysine residues within ORF0657t that are maximally shielded upon binding of ORF0657t to 2H2 mAb. The chemical labeling experiment described above identified K76, K257 and potentially K443 as being most protected upon binding of 2H2 mAb. Protection against chemical labeling is likely due to direct binding. However, it is possible that such protection could be due to binding in close proximity to the protected sites or by long range structural changes within ORFO657nI

In summary, the above described experiments provide clear evidence that the epitope within ORF0657t that is recognized by the 2H2 mAb is conformational. The fragment of ORF0657t that is recognized by 2H2 mAb has an N-terminus located between amino acids 1 and 115 of ORF0675t and a carboxyl terminus located between amino acids 323-357 of ORF0657t. Even though it can not be excluded that protection from chemical labeling upon binding of 2H2 mAb is influenced by long range structural changes, it is very likely that areas in close proximity to Lysine 76 and Lysine 275 participate in direct antibody interaction.

Example 9: 2H2 mAb Sequence Identification

Identification of the variable light (V_l) and variable heavy (V_h) sequences of hybridoma expressed 2H2 IgG was accomplished by combining the degenerative primer PCR /overlap extension cloning process for single chain variable fragments (scFv) assembly (Krebber *et al. JIM 201(1)*:35-55, 1997), with high throughput screening of soluble scFv fused to a human kappa light chain constant domain or scAb material via Biacore. This allowed for fine discrimination of mutations in V_l frameworks 1, 4 and V_h frameworks 1, 4 generated by the degenerative primer method.

Briefly, RNA material was purified from the hybridoma cell line using standard methods from a Total RNA Kit™ (Ambion Inc.). This material was then reverse transcribed to cDNA and utilized as template in PCR to amplify the variable regions. The conditions for the PCR amplification of the V_l and V_h chains was based upon the protocol described by Krebber *et al. JIM 201(1)*:35-55, 1997. The primers are designed such that a $(Gly4Ser)_4$ linker (SEQ ID NO: 32) is added which provides domains for a third PCR reaction in which the V_h and V_l are overlapped to create a V_l-$(Gly4Ser)_4$-V_h scFv.

The first set of PCR reactions to amplify the variable chains individually, were carried out in a volume of 100 µl containing 5 µl of the cDNA reaction, 2 µM each of the forward and reverse primer sets for amplification of V_l and V_h, and a high fidelity PCR master mix. The reactions were denatured for 4 minutes at 94°C followed by 30 cycles of 30 seconds at 94°C, 30 seconds at 50°C, 1

minute at 72°C, and finished at a final cycle of 5 minutes at 72°C. The full length PCR products were gel purified.

To construct the full length product a third PCR reaction was done to assemble to scFv from the amplified V_h and V_l material. In a volume of 100 µl approximately 20 ng each of V_h and V_l DNA and a high fidelity PCR master mix was denatured for 5 minutes at 94°C, followed by 3 cycles of 30 seconds at 94°C, 30 s at 60°C, and 30 seconds at 72°C in the absence of primers. The modified PCR primers, SEQ ID NO: 33 and SEQ ID NO: 34 were added at a final concentration of 1 µM, and 30 cycles of 30 seconds at 94°C, 1 minutes at 60°C, and 1 minute at 72°C were performed, followed by 7 minutes at 72°C. The expected full length scFv PCR products were gel purified.

The amplified scFv material was cloned into the MP16 soluble expression vector for scAb production (Hayhurst *et al.*, *JIM 276(1-2)*:185-196, 2003) and sequence analysis. A single restriction enzyme digest with Sfi1 was used for directional cloning into the MP16 vector. Clones with apparent full length variable heavy and variable light chains present were then expressed as scAbs in XL1-Blue cells and recovered from the periplasm using a standard osmotic shock procedure. Briefly, clones were grown at 37°C overnight in growth media containing 2% glucose and 100 µg/ml ampicillin in a 96 well format. 20 µl of the overnight culture was transferred to new media containing 0.1% glucose and 100 µg/ml ampicillin and grown until an OD_{600} of 0.6 was reached. The cells were induced for scAb expression by adding IPTG at a final concentration of 0.5 mM and incubated overnight while shaking at 150 rpm, at room temperature. The scAbs were purified from the cells using a Qiagen Ni-NTA superflow robotic procedure.

To analyze each scAb periplasmic preparation for binding activity to ORF0657t, a Biacore3000 surface plasmon resonance (SPR) instrument (Upsala, Sweden) was utilized. Standard EDC/NHS coupling was used to covalently mobilize approximately 250 resonance units of the 0657t antigen directly to the experimental flow cell surface of a CM5 sensor chip. A reference flow cell surface was activated and deactivated without coupling of protein. Each preparation was then run over the surface and association and dissociation of the scAb to antigen was measured. The surfaces were regenerated between runs by a single injection of 10 mM HCl for 20 seconds at a flow rate of 20 µl/min, followed by a 2 minute stabilization period. All samples were run in duplicate and buffer only runs were used as controls. After screening 95 clones, a clone was selected based on its binding activity. The final 2H2 clone chosen was based upon its similar affinity for ORF0657t as the original hybridoma prepared IgG material as well as comparative sequence analysis.

The amino sequence of the 2H2 V_h (SEQ ID NO: 20) and V_l (SEQ ID NO: 21) were as follows:

2H2 V_h Amino Acid Sequence (SEQ ID NO: 20)

```
  1   DVHLVESGPG LVAPSQNLSI TCTVSGFSLS RYGVHWVRQP PGKGLEWLGL
 51   IWAGGVTIYN STLMSRLSIS KDSSKSQVFL KMNSLQIDDT AIYYCAREAS
101   RDHYFDYWGQ GTTLTVSS
```

2H2 V_l Amino Acid Sequence (SEQ ID NO: 21)

```
  1   DIVMTQSPAI MSASPGEKIT MTCSASSSVS YIYWYQQKSG TSPKRWIYDT
 51   SKLASGVPFR FSGGGSGTSF SLTISSMEAE DAATYYCQQW SSNPLTFGAG
101   TKLEIK
```

The underlined portions are the CDR's. CDR's were identified based on the Kabat definition. The encoding nucleic acid sequence is provided by SEQ ID NO: 24 (V_h) and SEQ ID NO: 25 (V_l).

Example 10: *2H2 IgG Chimera Expression*

The variable regions for 2H2 mAb were cloned from mouse hybridoma as described in Example 9. The sequences for the variable regions were PCR amplified and DNA encoding the heavy chain variable regions were fused in-frame with DNA encoding the IgG1 constant region whereas DNA encoding the light chain variable region were fused in-frame with DNA encoding the kappa constant region. The cloning procedure for the resulting antibody expression vectors is described below.

The variable regions were PCR amplified. PCR reactions were carried out in a volume of 25 µl containing high fidelity PCR master mix, template volume 1 µl and forward and reverse primers: 1 µl each. PCR condition was 1 cycle of 94°C, 2 minutes, 25 cycles of 94°C, 1.5 minutes; 60°C, 1.5 minutes; 72°C, 1.5 minutes and 72°C, 7 minutes; 4°C until removed and cloned in-frame with leader sequence at the 5'-end and constant region at the 3'-end using In-Fusion strategy. The following primers were used: Light chain forward, 5'- ACAGATGCCAGATGCGATATTGTGATGACCCAGTCT (SEQ ID NO: 28); Light chain reverse, 5'- TGCAGCCACCGTACGTTTTATTTCCAGCTTGGTCCC (SEQ ID NO: 29); Heavy chain forward, 5'- ACAGGTGTCCACTCGGATGTGCACCTGGTGGAGTCA (SEQ ID NO: 30); and Heavy chain reverse, 5'- GCCCTTGGTGGATGCCGAGGAGACTGTGAGAGTGGT (SEQ ID NO: 31). The DNA sequences for all the clones were confirmed by sequencing.

The amino acid sequences deduced from DNA sequences are:

Mouse 2H2 Variable and Human Kappa Constant Region Amino Acid Sequence (SEQ ID NO: 22)

```
  1   DIVMTQSPAI MSASPGEKIT MTCSASSSVS YIYWYQQKSG TSPKRWIYDT
 51   SKLASGVPFR FSGGGSGTSF SLTISSMEAE DAATYYCQQW SSNPLTFGAG
101   TKLEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD
151   NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL
201   SSPVTKSFNR GEC
```

Mouse 2H2 Variable and Human IgG1 Constant Region Amino Acid Sequence (SEQ ID NO: 23)

1 DVHLVESGPG LVAPSQNLSI TCTVSGFSLS RYGVHWVRQP PGKGLEWLGL
51 IWAGGVTIYN STLMSRLSIS KDSSKSQVFL KMNSLQIDDT AIYYCAREAS
101 RDHYFDYWGQ GTTLTVSSAS TKGPSVFPLA PSSKSTSGGT AALGCLVKDY
151 FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTQTYI
201 CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAPELLGGPS VFLFPPKPKD
251 TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
301 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY
351 TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD
401 SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK

The variable regions are underlined.

The antibodies were expressed in 293EBNA monolayer cells. The plasmids were transfected using PEI based transfection reagents. The transfected cells were incubated in Opti-MEM serum free medium and the secreted antibodies were purified from medium using protein A/G affinity chromatography. The concentration of purified antibodies was determined by OD280 nm and the purity was measured by LabChip™ capillary electrophoresis.

The expression of both light and heavy chains was driven by human CMV promoter and bovine growth hormone polyadenylation signal. (Shiver *et al., Ann. N.Y. Acad. Sci., 772*:198-208, 1995.) The leader sequence in the front mediated the secretion of antibodies into the culture medium. The leader sequence for the heavy chain was MEWSWVFLFFLSVTTGVHS (SEQ ID NO: 26) and for the light chain was MSVPTQVLGLLLLWLTDARC (SEQ ID NO: 27). The expression vectors carry oriP from EBV viral genome for prolonged expression in 293EBNA cells and the bacterial sequences for kanamycin selection marker and replication origin in *E. coli*.

The antibodies were expressed in 293EBNA monolayer cells. The plasmids were transfected using PEI based transfection reagents. The transfected cells were incubated in Opti-MEM serum free medium and the secreted antibodies were purified from medium using protein A/G affinity chromatography. The concentration of purified antibodies was determined by OD280nm and the purity by LabChip capillary electrophoresis.

Example 11: Affinity Determination

Comparative analysis was performed on 2H2 mAb as hybridoma material, scAb and a chimeric antibody. 2H2 mAb V_h and V_l region were cloned and expressed as an IgG chimera as described in Example 10. scAb was cloned into the MP16 vector (Example 9), which produces a scFv with a Human Kappa chain tag fused to it. As further described below, the antigen affinity was not significantly different among the constructs.

To measure a 1:1 interaction between the binding domain and the antigen, the experimental set up on Biacore was modified depending on whether antibody fragment or full length IgG

was analyzed. For IgG measurements, the IgG was captured to the surface as ligand and ORF0657t was run as analyte. For antibody fragment analysis, ORF0657t was bound to the surface and the antibody fragment was run as the analyte. This demonstrated that the affinity of the original 2H2 mAb hybridoma material to the ORF0657t antigen shows no significant change upon recombinant cloning (Table 12). Data were acquired via surface plasmon resonance on a Biacore 3000; each analyte was run at multiple concentrations, with two replicates per concentration. Data were analyzed with BIAevaluation (Biacore, Inc.) with simultaneous fits of entire concentration series. Fit parameters are listed in Table 12.

Table 12

	On-rate ka (1/Ms)	Off-rate kd (1/s)	Affinity, KD	chi^2 global fit
2H2 murine IgG2b	6.10 E+04	2.01 E-03	33nM	0.902
2H2 scAb	4.91 E+04	1.91 E-03	39nM	0.429
2H2 IgG chimera	1.10 E+05	2.73 E-03	25nM	0.295

Example 12: ORF0657n Based Sequences

The highlighted amino acids (indicated by bold and underlying) present in SEQ ID NOs: 4-19 show amino acid alterations to ORF0657n:

0657n (SEQ ID NO: 1)

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKAVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNSRPIDFE
MKKENGEQQFYHYASSVKPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFSVSNGT
KAVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLKA
EYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

0657nC/e (SEQ ID NO: 2)

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKAVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNSRPIDFE
MKKENGEQQFYHYASSVKPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFSVSNGT
KAVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLKA
EYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKNLEHHHHHH

0657nH/y (SEQ ID NO: 3)

MAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVANAVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKA
TNNTYPILNQELREAIKNPAIKDKDHSAPNSRPIDFEMKKKDGTQQFYHYASSVKPARVIFTDSKPEIELGLQSGQF
WRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFSVSNGTKAVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEE
DYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLKAEYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDT
KYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVMETTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKT
LYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKSNKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDD
NKQLPSVEKENDASSESGKDKTPATKPTKGEVESSSTTPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSA
PLQKANIKNTNDGHTQSQNNKNTQENKAKS

SEQ ID NO: 4

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
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MKKKDGTQQFYHYASSVKPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFSVSNGT
KEVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLKA
EYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 5

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
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KEVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLKA
EYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 6

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
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MKKKDGTQQFYHYASSVEPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFSVSNGT
KAVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELEKIQDKLPEKLKA
EYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
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NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 7

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNWRPIDFE
MKKKDGTQQFYHYASSVEPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFSVSNGT
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EYKKKLEDTKKALAEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 8

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNWRPIDFE
MKKKDGTQQFYHYASSVEPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFSVSNGT
KEVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELEKIQDKLPEKLKA
EYKKKLEDTKKALAEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 9

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
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MKKKDGTQQFYHYASSVEPARVIFTKSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTDKDYAYIRFSVSNGT
KEVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELEKIQDKLPEKLKA
EYKKKLEDTKKALAEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 10

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNWRPIDFE
MKKKDGTQQFYHYASSVEPARVIFTKSKPEIELGLQSGSTWRKFEVYEGDKKLPIKLVSYDTDKDYAYIRFSVSNGT
KEVKIVSSTHFNNKEEKYDYTLMVFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELEKIQDKLPEKLKA
EYKKKLEDTKKALAEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST

TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 11

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNWRPIDFE
MKNDKGTQQFYHYASSVEPARVIFTKSKPIIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTDKDYAYIRFSVSNGT
KEVKIVSSTHFNNKEEKYDYTLMVFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELEKIQDKLPEKLKA
EYKKKLEDTKKALAEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 12

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNWRPIDFE
MKNDKGTQQFYHYASSVEPARVIFTKSKPIIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTDKDYAYIRFSVSNGT
KEVKIVSSTHFNNKEEKYDYTLMVFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELEKIQDKLPEKLKA
EYKKKLEQTKKALAEQVKSAITEFQNVQPTNEKMTDLQDAHYVVYESVENSESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 13

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNWRPIDFE
MKNDKGTQQFYHYASSVEPARVIFTKSKPIIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTDKDYAYIRFSVSNGT
KEVKIVSSTHFNNKEEKYDYTLMVFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELEKIQDKLPEKLKA
EYKKKLEQTKKALAEQVKSAITEFQNVQPTNEKMTDLQDAHYVVYESVENSESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGKRVRTISKDAKNNTRTIIFPYVEGKALYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 14

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIKDKEHSAPNSRPIDFE
MKKKDGTQQFYHYASSVKPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPVKLVSYDTVKDYAYIRFSVSNGT
KAVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKLQEKLPEKLKA
EYKKKLEDTKKALDEQVKSAVTEFQNVQPTNDKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME

TTNDDYWKDFMVEGQSVRTISKDAKNNTRTIIFPYIEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 15

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELRDAIKNPAIKDKEHSAPNSRPIDFE
MKKKDGTQQFYHYASTVKPARVIFTDTKPEIELGLQSGQFWRKFEVYEGDKKLPVKLVSYDSVKDYAYIRFSVSNGT
RAVKIVSSTHYNNKEEKYDYTLMEFAQPIYNSADKYKTEEDYKAEKLLAPYKKAKTLERQVYELNKLQDKLPEKLKA
EYKKKLDDTKKALDDQVKSAVTEFQNVQPTNEKMTDLQDTKYVVEESVENNESVMDTFVKHPIKTGMLNGKKYVVME
TTNDDYWKDFIVEGQRVRTVSKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 16

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIIDKDHSAPNSRPIDFE
MKKKDGTQQFYHYASSVKPARVIFTDSGPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFPVSNGT
KAVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLKA
EYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 17

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIIDKDHSAPNSRPIDFE
MKKKDGTQQFYHYASSVKPARVIFTDSGPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFPVSNGT
KAVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLKA
EYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESEENNESMMDTFVKHPIYTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 18

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIIDKDHSAPNSRPIDFE
MKKKDGTQQFYHYASSVKPARVIFTDSGPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFPVSNGT
KAVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKDEEDYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLKA
EYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESEENNESMMDTFVKHPIYTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 19

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
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DFEMKKKDGTQQFYHYASSVKPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFSVS
NGTKAVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEK
LKAEYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYM
VMETTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDVDKEAFTKA
NTDKSNKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEV
ESSSTTPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLP
QTGEESNKDMTLPLMALLALSSIVAFVLPRKRKN

Other embodiments are within the following claims. While several embodiments have been shown and described, various modifications may be made without departing from the spirit and scope of the present invention.

WHAT IS CLAIMED IS:

1. An isolated antigen binding protein comprising a first variable region and a second variable region, wherein said binding protein binds to a target region selected from the group consisting of: mAb IG3.BD4 target region, mAb 2H2.BE11 target region, mAb 13C7.BC1, and mAb 13G11.BF3 target region.

2. The binding protein of claim 1, wherein said target region is the mAb 2H2.BE11 target region and said first variable region is a V_h region comprising:

a first V_h CDR comprising amino acids 36-45 of SEQ ID NO: 20 or a sequence differing from amino acids 36-45 by one amino acid;

a second V_h CDR comprising amino acids 50-65 of SEQ ID NO: 20 or a sequence differing from amino acids 50-65 by one amino acid; and

a third V_h CDR comprising amino acids 98-107 of SEQ ID NO: 20 or a sequence differing from amino acids 98-107 by one amino acid.

3. The binding protein of claim 2, wherein said second variable region is a V_l region comprising:

a first V_l CDR comprising amino acids 24-33 of SEQ ID NO: 21 or a sequence differing from amino acids 24-33 by one amino acid;

a second V_l CDR comprising amino acids 49-55 of SEQ ID NO: 21 or a sequence differing from amino acids 49-55 by one amino acid; and

a third V_l CDR comprising amino acids 88-96 of SEQ ID NO: 21 or a sequence differing from amino acids 88-96 by one amino acid.

4. The binding protein of claim 3, wherein said binding protein is an antibody.

5. The binding protein of claim 4, wherein said antibody is a monoclonal antibody.

6. The binding protein of claim 4, wherein said V_h region is either SEQ ID NO: 20, a humanized SEQ ID NO: 20 , or a de-immunized SEQ ID NO: 20; and said V_l region is either SEQ ID NO: 21, a humanized SEQ ID NO: 21, or a de-immunized SEQ ID NO: 21.

7. The binding protein of claim 6, wherein said binding protein is an antibody comprising (a) a heavy chain comprising said V_h region, and a human hinge, CH_1, CH_2, and CH_3 regions from an IgG_1, IgG_2, IgG_3 or IgG_4 subtype; and (b) a light chain comprising said V_l region, and either a human kappa C_L or human lambda C_L.

8. The binding protein of claim 3, wherein

said V_h region comprises said first V_h CDR consisting of amino acids 36-45 of SEQ ID NO: 20, said second V_h CDR consisting of amino acids 50-65 of SEQ ID NO: 20, and said third V_h CDR consisting of amino acids 98-107 of SEQ ID NO: 20 and;

said first V_l region comprises said first V_l CDR consisting of amino acids 24-33 of SEQ ID NO: 21, said second V_l CDR consisting of amino acids 49-55 of SEQ ID NO: 21, and said third V_l CDR consisting of amino acids 88-96 of SEQ ID NO: 21.

9. The binding protein of claim 8, wherein said binding protein is an antibody.

10. The binding protein of claim 9, wherein said antibody is a monoclonal antibody.

11. The binding protein of claim 9, wherein said V_h region is either SEQ ID NO: 20, a humanized SEQ ID NO: 20, or a de-immunized SEQ ID NO: 20; and said V_l region is either SEQ ID NO: 21, a humanized SEQ ID NO: 21, or a de-immunized SEQ ID NO: 21.

12. The binding protein of claim 8, wherein said binding protein is an antibody comprising (a) a heavy chain comprising said V_h region, and a human hinge, CH_1, CH_2, and CH_3 regions from an IgG_1, IgG_2, IgG_3 or IgG_4 subtype; and (b) a light chain comprising said V_l region, and either a human kappa C_L or human lambda C_L.

13. The binding protein of claim 12, wherein said heavy chain consists essentially of the amino acid sequence of SEQ ID NO: 22; and said light chain consists essentially of the amino acid sequence of SEQ ID NO: 23.

14. A nucleic acid comprising a recombinant gene comprising a nucleotide sequence encoding an antibody variable region that binds to a target region selected from the group consisting of: mAb IG3.BD4 target region, mAb 2H2.BE11 target region, mAb 13C7.BC1, and mAb 13G11.BF3 target region.

15. The nucleic acid of claim 14, wherein said target region is the mAb 2H2.BE11 target region and said variable region is a V_h region comprising:

a first V_h CDR comprising amino acids 36-45 of SEQ ID NO: 20 or a sequence differing from amino acids 36-45 by one amino acid;

a second V_h CDR comprising amino acids 50-65 of SEQ ID NO: 20 or a sequence differing from amino acids 50-65 by one amino acids; and

a third V_h CDR comprising amino acids 98-107 of SEQ ID NO: 20 or a sequence differing from amino acids 98-107 by one amino acid.

16. The nucleic acid of claim 15, wherein said V_h region comprises said first V_h CDR consisting of amino acids 36-45 of SEQ ID NO: 20; said second V_h CDR consisting of amino acids 50-65 of SEQ ID NO: 20; and said third V_h CDR consisting of amino acids 98-107 of SEQ ID NO: 20.

17. The nucleic acid of claim 14, wherein said variable region is either SEQ ID NO: 20, a humanized SEQ ID NO: 20, or a de-immunized SEQ ID NO: 20.

18. The nucleic acid of claim 17, wherein said recombinant gene encodes an antibody heavy chain comprising said variable region, a human hinge, and CH_1, CH_2, and CH_3 regions from an IgG_1, IgG_2, IgG_3 or IgG_4 subtype.

19. The nucleic acid of claim 14, wherein said variable region is a V_l region comprising:

a first V_l CDR comprising amino acids 24-33 of SEQ ID NO: 21 or a sequence differing from amino acids 24-33 by one amino acid;

a second V_l CDR comprising amino acids 49-55 of SEQ ID NO: 21 or a sequence differing from amino acids 49-55 by one amino acid; and

a third V_l CDR comprising amino acids 88-96 of SEQ ID NO: 21 or a sequence differing from amino acids 88-96 by one amino acid.

20. The nucleic acid of claim 19, wherein said first V_l region comprises said first V_l CDR consisting of amino acids 24-33 of SEQ ID NO: 21, said second V_l CDR consisting of amino acids 49-55 of SEQ ID NO: 21, and said third V_l CDR consisting of amino acids 88-96 of SEQ ID NO: 21.

21. The nucleic acid of claim 14, wherein said variable region is either SEQ ID NO: 21, a humanized SEQ ID NO: 21, or a de-immunized SEQ ID NO: 21.

22. The nucleic acid of claim 17, wherein said recombinant gene encodes an antibody light chain comprising said variable region and a human kappa or lambda C_L.

23. A recombinant cell comprising one or more nucleic acids of any one of claims 14-22.

24. The recombinant cell of claim 23, wherein said cell comprises both the nucleic acid of claim 18 and the nucleic acid of claim 22.

25. A method of producing protein comprising an antibody variable region comprising the steps of:

a) growing the recombinant cell of claim 23 under conditions wherein said protein is expressed; and

b) purifying said protein.

26. A method of producing protein comprising an antibody variable region comprising the steps of:

a) growing the recombinant cell of claim 24 under conditions wherein said protein is expressed; and

b) purifying said protein.

27. A pharmaceutical composition comprising the binding protein of any one of claims 1-13 and a pharmaceutically acceptable carrier.

28. A method of detecting the presence of an OFR0657n antigen in a solution or on a cell comprising the steps of: (a) providing the binding protein of any one of claims 1-13 to said solution or said cell; and (b) measuring the ability of said binding protein to bind to said antigen present in said solution or to said cell.

29. A method of treating against an *S. aureus* infection in a patient comprising the step of administering to said patient an effective amount of the binding protein of any one of claims 1-13.

30. The method of claim 29, wherein said antigen binding protein is administered in conjunction with surgery or a foreign body implant.

31. A cell line producing a protein that is either mAb 1G3.B3, mAb 2H2.B8, mAb 13C7.D12, or mAb 13G11.C11, or that competes with either mAb IG3.B3., mAb 2H2.B8, mAb 13C7.D12, or mAb 13G11.C11 for binding to ORF0657n.

32. The cell line of claim 31, wherein said line is either ATCC No: PTA-7124, ATCC No: PTA-7125, ATCC No: PTA-7126 or ATCC No: PTA-7127.

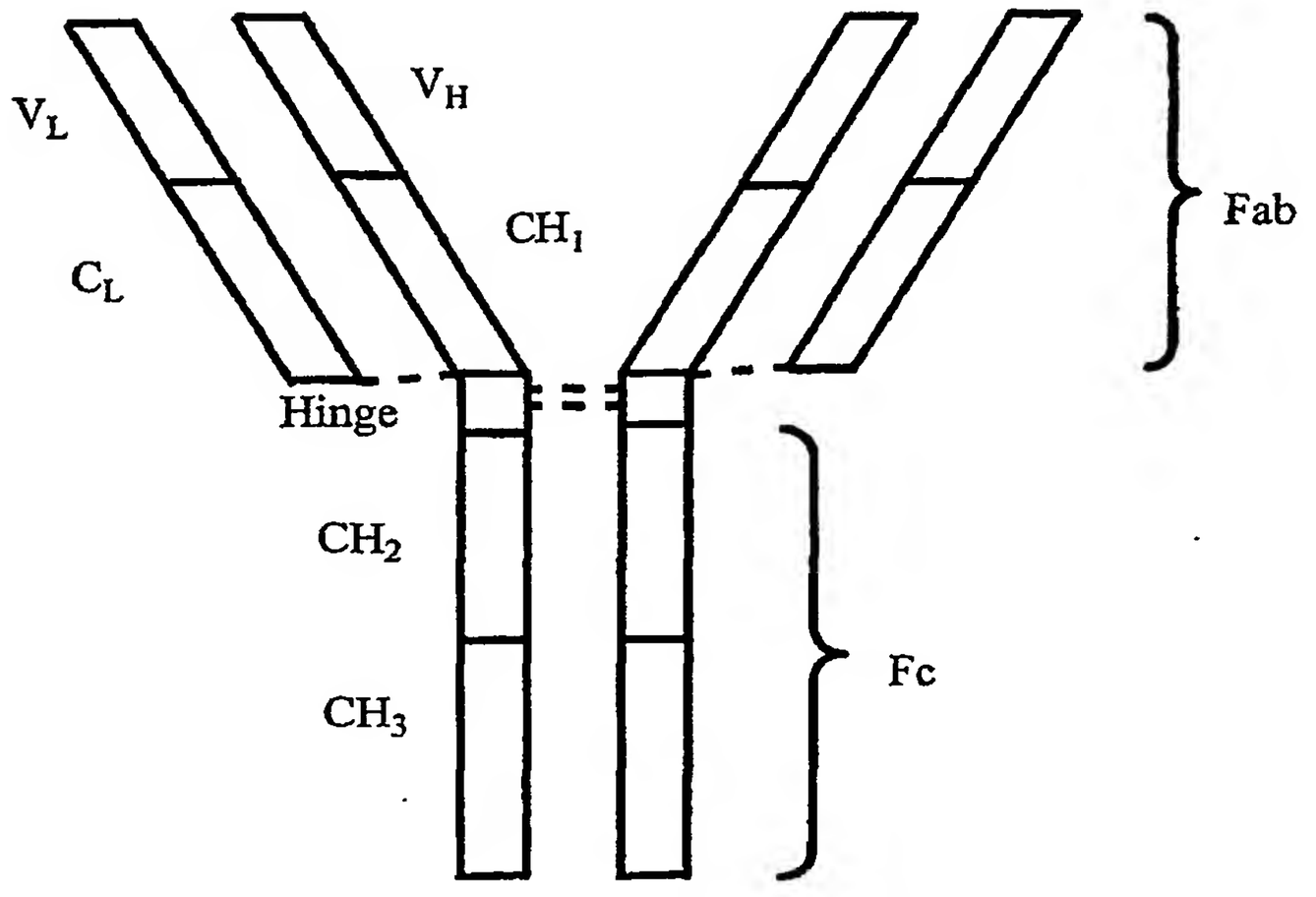

FIG. 1

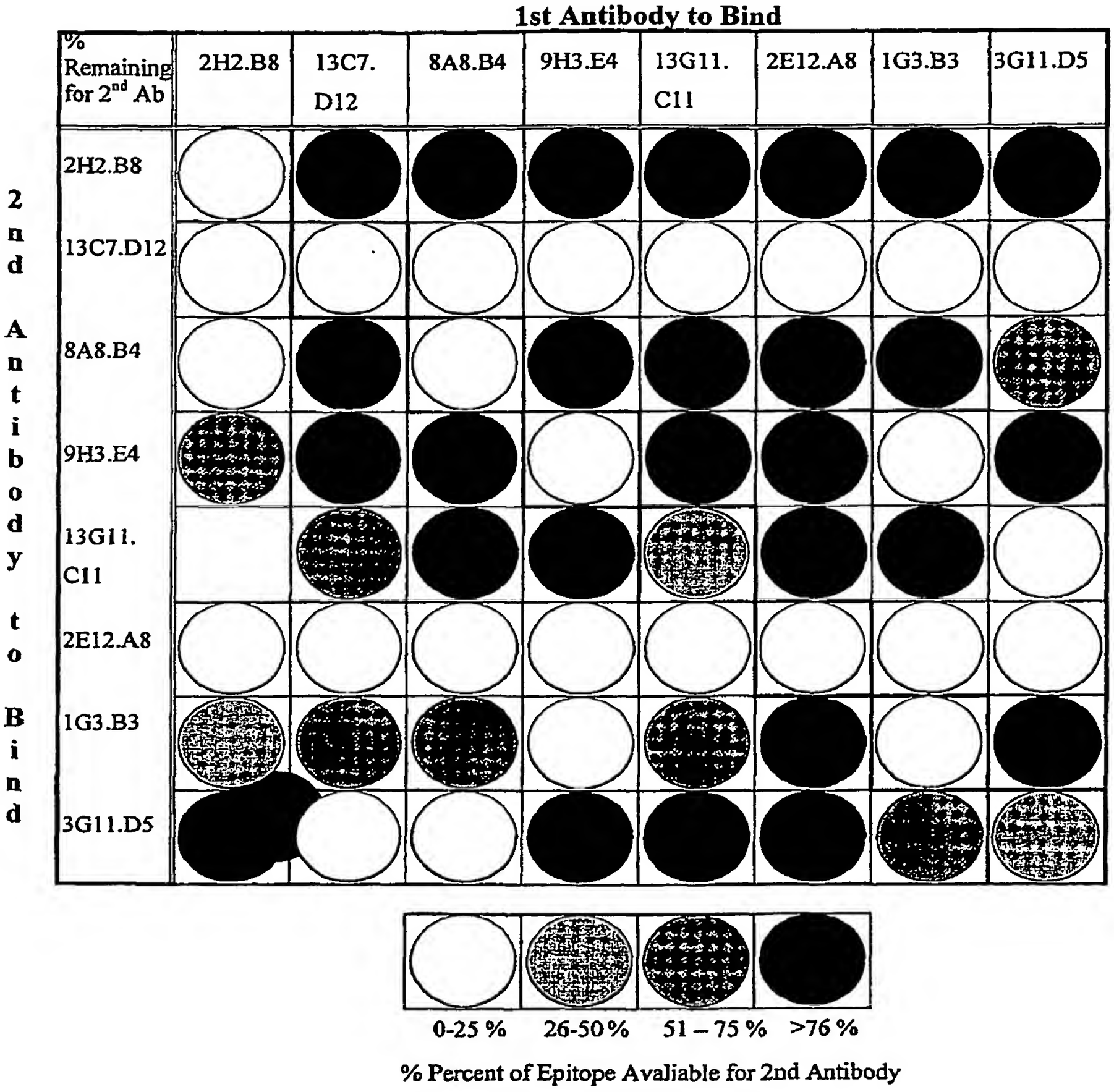
1st Antibody to Bind
% Remaining for 2nd Ab
2H2.B8
13C7. D12
8A8.B4
9H3.E4
13G11. C11
2E12.A8
1G3.B3
3G11.D5
2nd Antibody to Bind
0-25 %
26-50 %
51 – 75 %
>76 %
% Percent of Epitope Avaliable for 2nd Antibody

FIG. 2

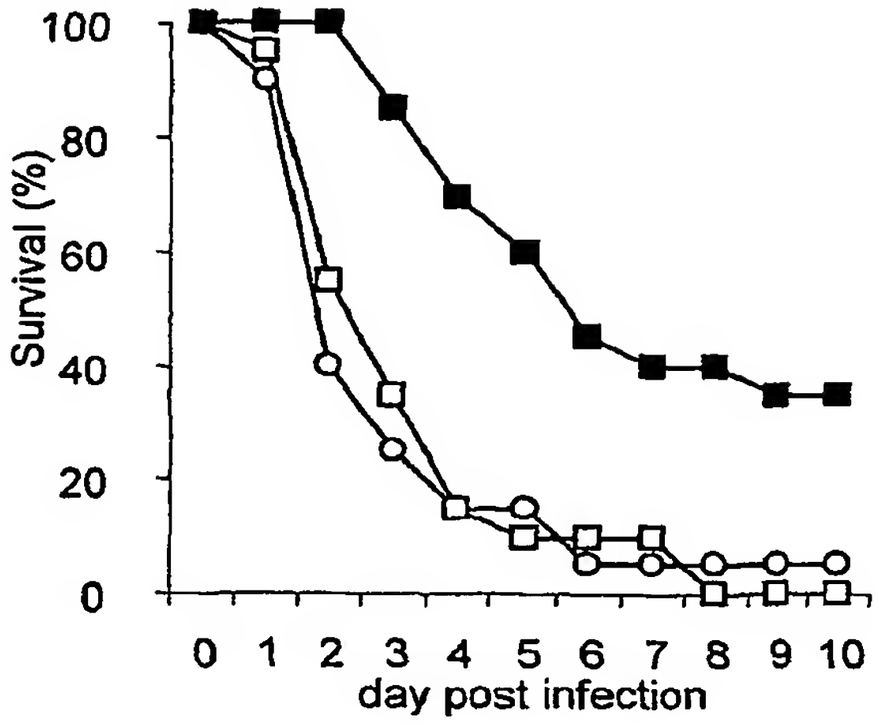

FIG. 3A

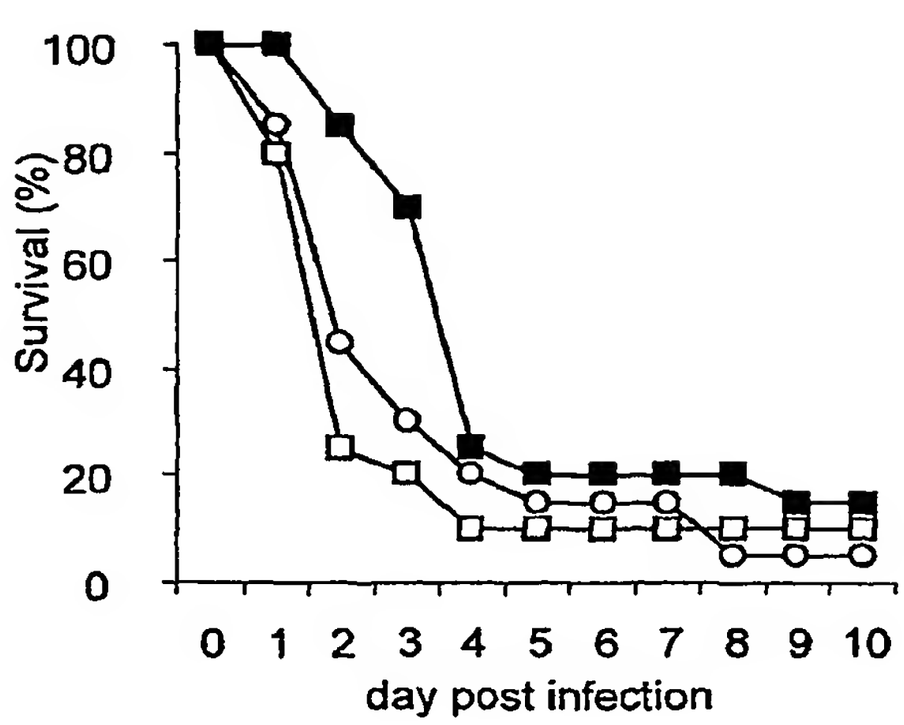

FIG. 3B

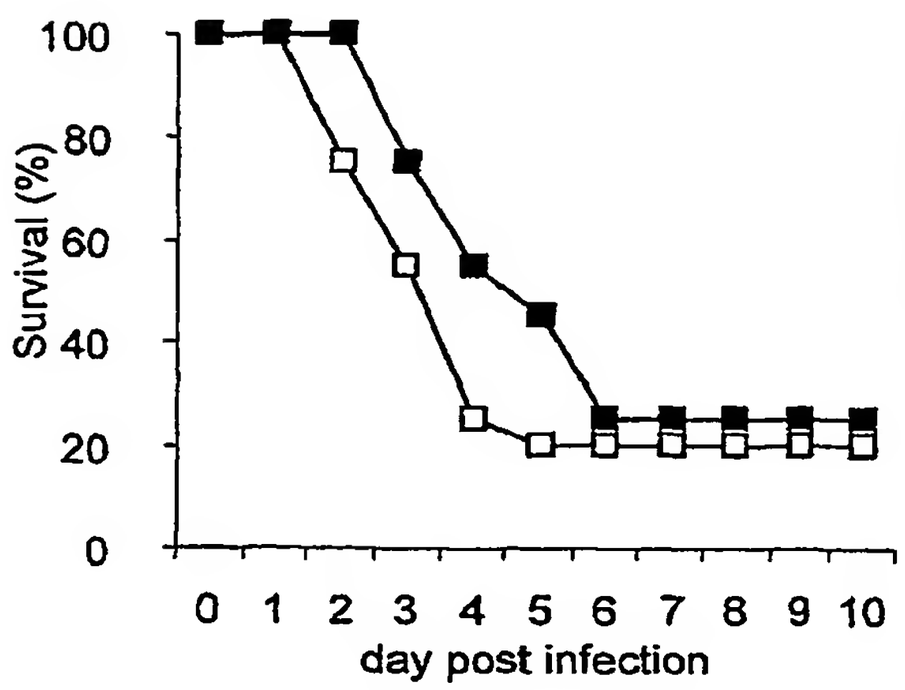

FIG. 3C

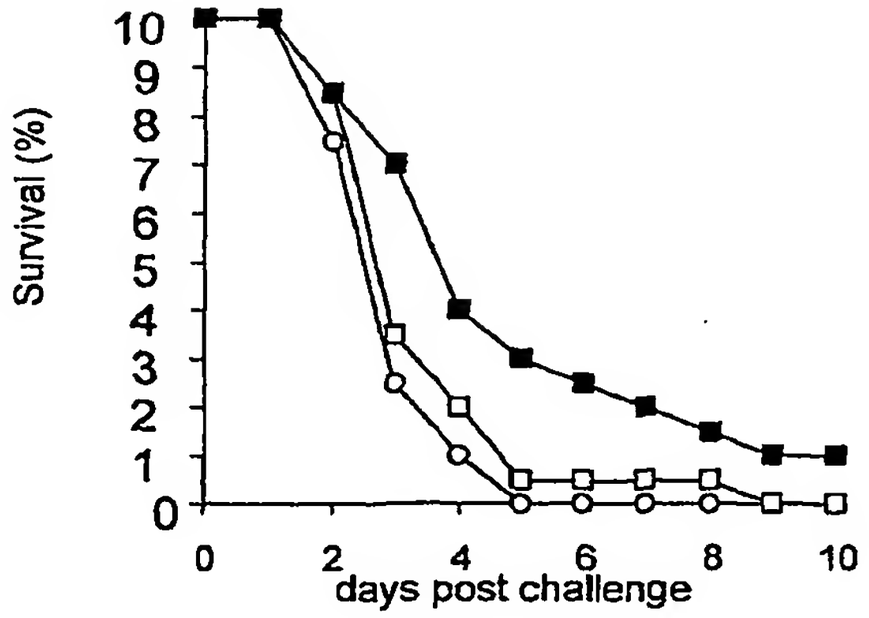

FIG. 4A

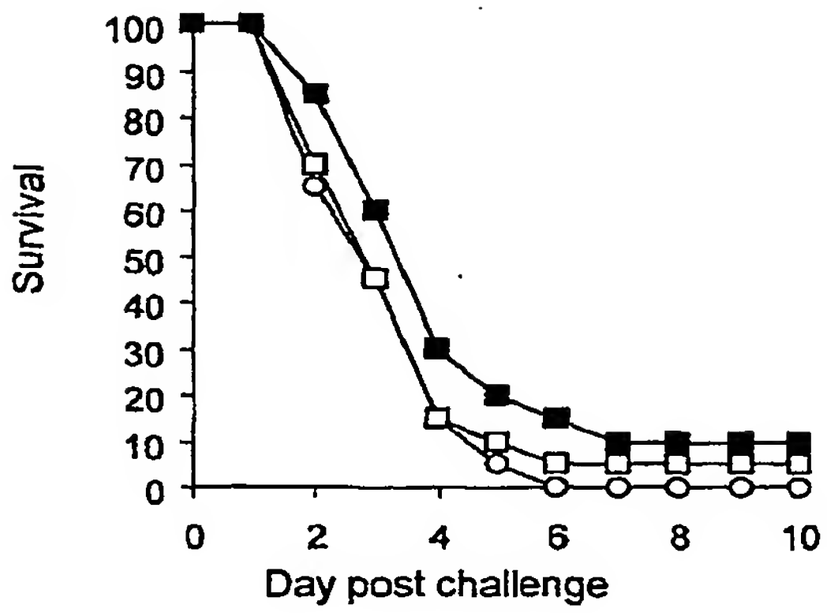

FIG. 4B

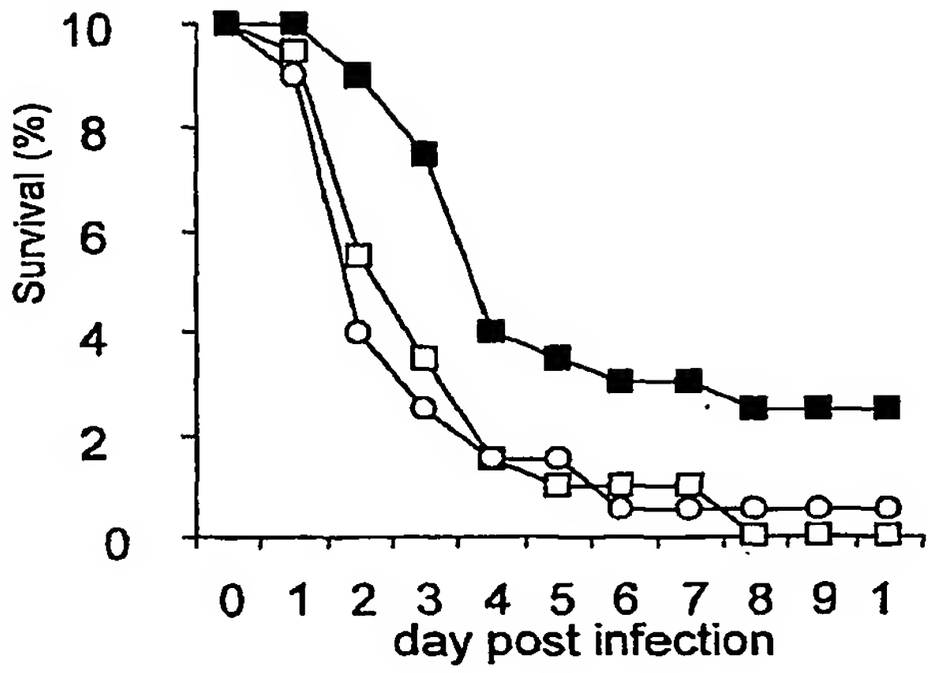

FIG. 5A

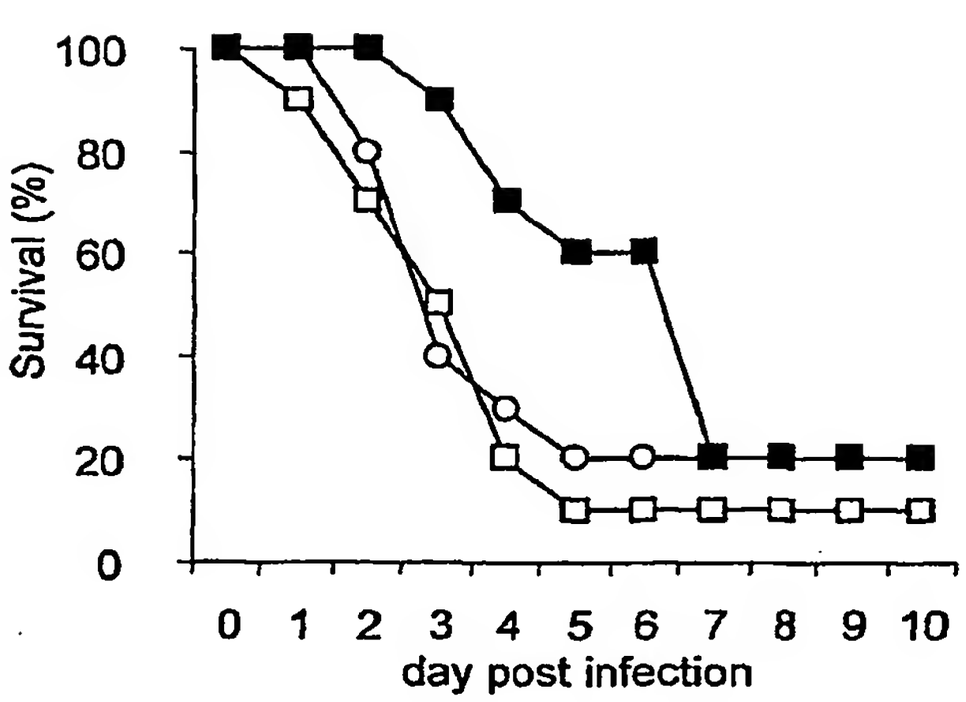

FIG. 5B

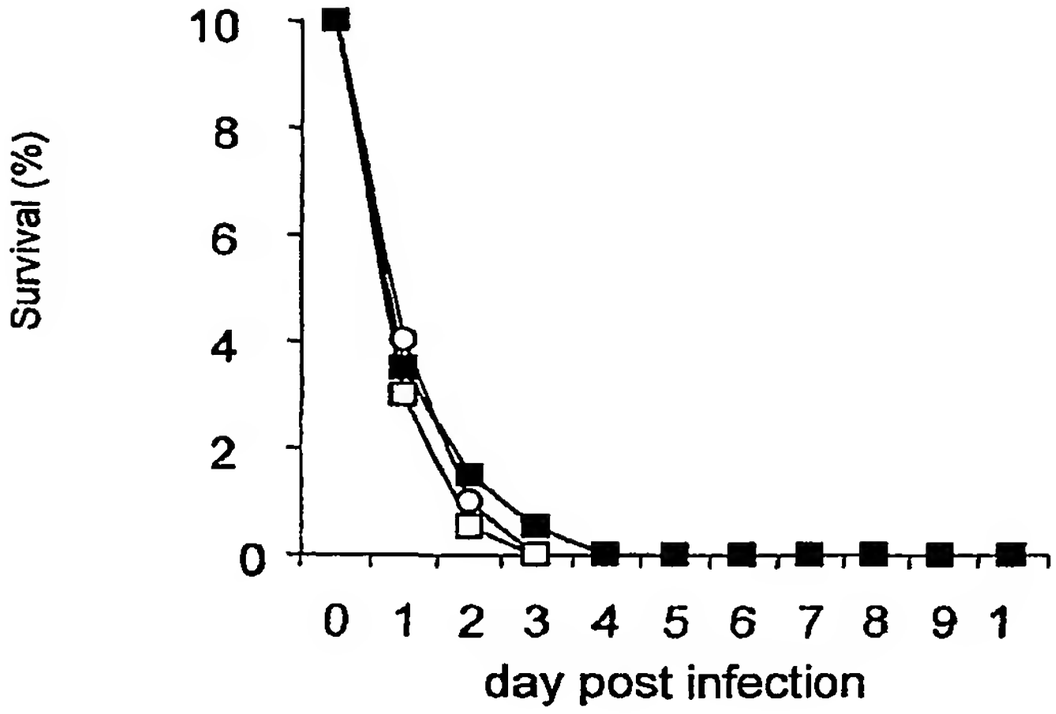

FIG. 5C

SEQUENCE LISTING

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<150> 60/763,023
<151> 2006-01-27

<160> 34

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 645
<212> PRT
<213> Staphylococcus aureus

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Asn	Thr	Glu	Ala	Gln	Pro	Lys	Thr	Glu	Ala	Val	Ala	Ser	Pro	Thr	Thr
	50					55					60				
Thr	Ser	Glu	Lys	Ala	Pro	Glu	Thr	Lys	Pro	Val	Ala	Asn	Ala	Val	Ser
65					70					75					80
Val	Ser	Asn	Lys	Glu	Val	Glu	Ala	Pro	Thr	Ser	Glu	Thr	Lys	Glu	Ala
				85					90					95	
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			100					105					110		
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Glu	Gln	Gln	Phe	Tyr	His	Tyr	Ala	Ser	Ser	Val	Lys	Pro	Ala	Arg	Val
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Gln	Phe	Trp	Arg	Lys	Phe	Glu	Val	Tyr	Glu	Gly	Asp	Lys	Lys	Leu	Pro
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225					230					235					240
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    290                 295                 300
Lys Leu Lys Ala Glu Tyr Lys Lys Lys Leu Glu Asp Thr Lys Lys Ala
305                 310                 315                 320
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Pro Thr Asn Glu Lys Met Thr Asp Leu Gln Asp Thr Lys Tyr Val Val
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Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr Asp Lys Ser Asn Lys
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                485                 490                 495
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        515                 520                 525
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    530                 535                 540
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545                 550                 555                 560
Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly Ser Ser Glu Ala Lys
                565                 570                 575
Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
            580                 585                 590
His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln Glu Asn Lys Ala Lys
        595                 600                 605
Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro
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Leu Met Ala Leu Leu Ala Leu Ser Ser Ile Val Ala Phe Val Leu Pro
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			20					25					30		
Met	Ser	Asn	Gly	Glu	Ala	Gln	Ala	Ala	Ala	Glu	Glu	Thr	Gly	Gly	Thr
		35					40					45			
Asn	Thr	Glu	Ala	Gln	Pro	Lys	Thr	Glu	Ala	Val	Ala	Ser	Pro	Thr	Thr
	50					55					60				
Thr	Ser	Glu	Lys	Ala	Pro	Glu	Thr	Lys	Pro	Val	Ala	Asn	Ala	Val	Ser
65					70					75					80
Val	Ser	Asn	Lys	Glu	Val	Glu	Ala	Pro	Thr	Ser	Glu	Thr	Lys	Glu	Ala
				85					90					95	
Lys	Glu	Val	Lys	Glu	Val	Lys	Ala	Pro	Lys	Glu	Thr	Lys	Ala	Val	Lys
			100					105					110		
Pro	Ala	Ala	Lys	Ala	Thr	Asn	Asn	Thr	Tyr	Pro	Ile	Leu	Asn	Gln	Glu
		115					120					125			
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	130					135					140				
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145					150					155					160
Glu	Gln	Gln	Phe	Tyr	His	Tyr	Ala	Ser	Ser	Val	Lys	Pro	Ala	Arg	Val
				165					170					175	
Ile	Phe	Thr	Asp	Ser	Lys	Pro	Glu	Ile	Glu	Leu	Gly	Leu	Gln	Ser	Gly
			180					185					190		
Gln	Phe	Trp	Arg	Lys	Phe	Glu	Val	Tyr	Glu	Gly	Asp	Lys	Lys	Leu	Pro
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	210					215					220				
Phe	Ser	Val	Ser	Asn	Gly	Thr	Lys	Ala	Val	Lys	Ile	Val	Ser	Ser	Thr
225					230					235					240
His	Phe	Asn	Asn	Lys	Glu	Glu	Lys	Tyr	Asp	Tyr	Thr	Leu	Met	Glu	Phe
				245					250					255	
Ala	Gln	Pro	Ile	Tyr	Asn	Ser	Ala	Asp	Lys	Phe	Lys	Thr	Glu	Glu	Asp
			260					265					270		
Tyr	Lys	Ala	Glu	Lys	Leu	Leu	Ala	Pro	Tyr	Lys	Lys	Ala	Lys	Thr	Leu
		275					280					285			
Glu	Arg	Gln	Val	Tyr	Glu	Leu	Asn	Lys	Ile	Gln	Asp	Lys	Leu	Pro	Glu
	290					295					300				
Lys	Leu	Lys	Ala	Glu	Tyr	Lys	Lys	Lys	Leu	Glu	Asp	Thr	Lys	Lys	Ala
305					310					315					320
Leu	Asp	Glu	Gln	Val	Lys	Ser	Ala	Ile	Thr	Glu	Phe	Gln	Asn	Val	Gln
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Pro	Thr	Asn	Glu	Lys	Met	Thr	Asp	Leu	Gln	Asp	Thr	Lys	Tyr	Val	Val
			340					345					350		
Tyr	Glu	Ser	Val	Glu	Asn	Asn	Glu	Ser	Met	Met	Asp	Thr	Phe	Val	Lys
		355					360					365			
His	Pro	Ile	Lys	Thr	Gly	Met	Leu	Asn	Gly	Lys	Lys	Tyr	Met	Val	Met
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Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu Ala Thr Pro Ala Thr
465                 470                 475                 480
Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys Glu Ser Gln Lys Gln
                485                 490                 495
Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro Ser Val Glu Lys Glu
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Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly Ser Ser Glu Ala Lys
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Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
            580                 585                 590
His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln Glu Asn Lys Ala Lys
        595                 600                 605
Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro
    610                 615                 620
Leu Met Ala Leu Leu Ala Leu Ser Ser Ile Val Ala Phe Val Leu Pro
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        35                  40                  45
Pro Thr Ser Glu Thr Lys Glu Ala Lys Glu Val Lys Glu Val Lys Ala
    50                  55                  60
Pro Lys Glu Thr Lys Glu Val Lys Pro Ala Ala Lys Ala Thr Asn Asn
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Thr Tyr Pro Ile Leu Asn Gln Glu Leu Arg Glu Ala Ile Lys Asn Pro
                85                  90                  95
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Ile Glu Leu Gly Leu Gln Ser Gly Gln Phe Trp Arg Lys Phe Glu Val
145                 150                 155                 160
Tyr Glu Gly Asp Lys Lys Leu Pro Ile Lys Leu Val Ser Tyr Asp Thr
                165                 170                 175
Val Lys Asp Tyr Ala Tyr Ile Arg Phe Ser Val Ser Asn Gly Thr Lys
            180                 185                 190
Ala Val Lys Ile Val Ser Ser Thr His Phe Asn Asn Lys Glu Glu Lys
        195                 200                 205
Tyr Asp Tyr Thr Leu Met Glu Phe Ala Gln Pro Ile Tyr Asn Ser Ala
    210                 215                 220
Asp Lys Phe Lys Thr Glu Glu Asp Tyr Lys Ala Glu Lys Leu Leu Ala
225                 230                 235                 240
Pro Tyr Lys Lys Ala Lys Thr Leu Glu Arg Gln Val Tyr Glu Leu Asn
                245                 250                 255
Lys Ile Gln Asp Lys Leu Pro Glu Lys Leu Lys Ala Glu Tyr Lys Lys
            260                 265                 270
Lys Leu Glu Asp Thr Lys Lys Ala Leu Asp Glu Gln Val Lys Ser Ala
        275                 280                 285
Ile Thr Glu Phe Gln Asn Val Gln Pro Thr Asn Glu Lys Met Thr Asp
    290                 295                 300
Leu Gln Asp Thr Lys Tyr Val Val Tyr Glu Ser Val Glu Asn Asn Glu
305                 310                 315                 320
Ser Met Met Asp Thr Phe Val Lys His Pro Ile Lys Thr Gly Met Leu
                325                 330                 335
Asn Gly Lys Lys Tyr Met Val Met Glu Thr Thr Asn Asp Asp Tyr Trp
            340                 345                 350
Lys Asp Phe Met Val Glu Gly Gln Arg Val Arg Thr Ile Ser Lys Asp
        355                 360                 365
Ala Lys Asn Asn Thr Arg Thr Ile Ile Phe Pro Tyr Val Glu Gly Lys
    370                 375                 380
Thr Leu Tyr Asp Ala Ile Val Lys Val His Val Lys Thr Ile Asp Tyr
385                 390                 395                 400
Asp Gly Gln Tyr His Val Arg Ile Val Asp Lys Glu Ala Phe Thr Lys
                405                 410                 415
Ala Asn Thr Asp Lys Ser Asn Lys Lys Glu Gln Gln Asp Asn Ser Ala
            420                 425                 430
Lys Lys Glu Ala Thr Pro Ala Thr Pro Ser Lys Pro Thr Pro Ser Pro
        435                 440                 445
Val Glu Lys Glu Ser Gln Lys Gln Asp Ser Gln Lys Asp Asp Asn Lys
    450                 455                 460
Gln Leu Pro Ser Val Glu Lys Glu Asn Asp Ala Ser Ser Glu Ser Gly
465                 470                 475                 480
Lys Asp Lys Thr Pro Ala Thr Lys Pro Thr Lys Gly Glu Val Glu Ser
                485                 490                 495
Ser Ser Thr Thr Pro Thr Lys Val Val Ser Thr Thr Gln Asn Val Ala
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Lys Pro Thr Thr Ala Ser Ser Lys Thr Thr Lys Asp Val Val Gln Thr
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        35                  40                  45
Asn Thr Glu Ala Gln Pro Lys Thr Glu Ala Val Ala Ser Pro Thr Thr
    50                  55                  60
Thr Ser Glu Lys Ala Pro Glu Thr Lys Pro Val Ala Asn Ala Val Ser
65                  70                  75                  80
Val Ser Asn Lys Glu Val Glu Ala Pro Thr Ser Glu Thr Lys Glu Ala
                85                  90                  95
Lys Glu Val Lys Glu Val Lys Ala Pro Lys Glu Thr Lys Glu Val Lys
            100                 105                 110
Pro Ala Ala Lys Ala Thr Asn Asn Thr Tyr Pro Ile Leu Asn Gln Glu
        115                 120                 125
Leu Arg Glu Ala Ile Lys Asn Pro Ala Ile Lys Asp Lys Asp His Ser
    130                 135                 140
Ala Pro Asn Ser Arg Pro Ile Asp Phe Glu Met Lys Lys Lys Asp Gly
145                 150                 155                 160
Thr Gln Gln Phe Tyr His Tyr Ala Ser Ser Val Lys Pro Ala Arg Val
                165                 170                 175
Ile Phe Thr Asp Ser Lys Pro Glu Ile Glu Leu Gly Leu Gln Ser Gly
            180                 185                 190
Gln Phe Trp Arg Lys Phe Glu Val Tyr Glu Gly Asp Lys Lys Leu Pro
        195                 200                 205
Ile Lys Leu Val Ser Tyr Asp Thr Val Lys Asp Tyr Ala Tyr Ile Arg
    210                 215                 220
Phe Ser Val Ser Asn Gly Thr Lys Glu Val Lys Ile Val Ser Ser Thr
225                 230                 235                 240
His Phe Asn Asn Lys Glu Glu Lys Tyr Asp Tyr Thr Leu Met Glu Phe
                245                 250                 255
Ala Gln Pro Ile Tyr Asn Ser Ala Asp Lys Phe Lys Thr Glu Glu Asp
            260                 265                 270
Tyr Lys Ala Glu Lys Leu Leu Ala Pro Tyr Lys Lys Ala Lys Thr Leu
        275                 280                 285
Glu Arg Gln Val Tyr Glu Leu Asn Lys Ile Gln Asp Lys Leu Pro Glu
    290                 295                 300
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Lys Leu Lys Ala Glu Tyr Lys Lys Lys Leu Glu Asp Thr Lys Lys Ala
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Leu Asp Glu Gln Val Lys Ser Ala Ile Thr Glu Phe Gln Asn Val Gln
                325                 330                 335
Pro Thr Asn Glu Lys Met Thr Asp Leu Gln Asp Thr Lys Tyr Val Val
            340                 345                 350
Tyr Glu Ser Val Glu Asn Asn Glu Ser Met Met Asp Thr Phe Val Lys
        355                 360                 365
His Pro Ile Lys Thr Gly Met Leu Asn Gly Lys Lys Tyr Met Val Met
    370                 375                 380
Glu Thr Thr Asn Asp Asp Tyr Trp Lys Asp Phe Met Val Glu Gly Gln
385                 390                 395                 400
Arg Val Arg Thr Ile Ser Lys Asp Ala Lys Asn Asn Thr Arg Thr Ile
                405                 410                 415
Ile Phe Pro Tyr Val Glu Gly Lys Thr Leu Tyr Asp Ala Ile Val Lys
            420                 425                 430
Val His Val Lys Thr Ile Asp Tyr Asp Gly Gln Tyr His Val Arg Ile
        435                 440                 445
Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr Asp Lys Ser Asn Lys
    450                 455                 460
Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu Ala Thr Pro Ala Thr
465                 470                 475                 480
Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys Glu Ser Gln Lys Gln
                485                 490                 495
Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro Ser Val Glu Lys Glu
            500                 505                 510
Asn Asp Ala Ser Ser Glu Ser Gly Lys Asp Lys Thr Pro Ala Thr Lys
        515                 520                 525
Pro Thr Lys Gly Glu Val Glu Ser Ser Ser Thr Thr Pro Thr Lys Val
    530                 535                 540
Val Ser Thr Thr Gln Asn Val Ala Lys Pro Thr Thr Ala Ser Ser Lys
545                 550                 555                 560
Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly Ser Ser Glu Ala Lys
                565                 570                 575
Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
            580                 585                 590
His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln Glu Asn Lys Ala Lys
        595                 600                 605
Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro
    610                 615                 620
Leu Met Ala Leu Leu Ala Leu Ser Ser Ile Val Ala Phe Val Leu Pro
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            20                  25                  30
Met Ser Asn Gly Glu Ala Gln Ala Ala Ala Glu Glu Thr Gly Gly Thr
        35                  40                  45
Asn Thr Glu Ala Gln Pro Lys Thr Glu Ala Val Ala Ser Pro Thr Thr
    50                  55                  60
Thr Ser Glu Lys Ala Pro Glu Thr Lys Pro Val Ala Asn Ala Val Ser
65                  70                  75                  80
Val Ser Asn Lys Glu Val Glu Ala Pro Thr Ser Glu Thr Lys Glu Ala
                85                  90                  95
Lys Glu Val Lys Glu Val Lys Ala Pro Lys Glu Thr Lys Glu Val Lys
            100                 105                 110
Pro Ala Ala Lys Ala Thr Asn Asn Thr Tyr Pro Ile Leu Asn Gln Glu
        115                 120                 125
Leu Arg Glu Ala Ile Lys Asn Pro Ala Ile Lys Asp Lys Asp His Ser
    130                 135                 140
Ala Pro Asn Trp Arg Pro Ile Asp Phe Glu Met Lys Lys Lys Asp Gly
145                 150                 155                 160
Thr Gln Gln Phe Tyr His Tyr Ala Ser Ser Val Glu Pro Ala Arg Val
                165                 170                 175
Ile Phe Thr Asp Ser Lys Pro Glu Ile Glu Leu Gly Leu Gln Ser Gly
            180                 185                 190
Gln Phe Trp Arg Lys Phe Glu Val Tyr Glu Gly Asp Lys Lys Leu Pro
        195                 200                 205
Ile Lys Leu Val Ser Tyr Asp Thr Val Lys Asp Tyr Ala Tyr Ile Arg
    210                 215                 220
Phe Ser Val Ser Asn Gly Thr Lys Glu Val Lys Ile Val Ser Ser Thr
225                 230                 235                 240
His Phe Asn Asn Lys Glu Glu Lys Tyr Asp Tyr Thr Leu Met Glu Phe
                245                 250                 255
Ala Gln Pro Ile Tyr Asn Ser Ala Asp Lys Phe Lys Thr Glu Glu Asp
            260                 265                 270
Tyr Lys Ala Glu Lys Leu Leu Ala Pro Tyr Lys Lys Ala Lys Thr Leu
        275                 280                 285
Glu Arg Gln Val Tyr Glu Leu Asn Lys Ile Gln Asp Lys Leu Pro Glu
    290                 295                 300
Lys Leu Lys Ala Glu Tyr Lys Lys Lys Leu Glu Asp Thr Lys Lys Ala
305                 310                 315                 320
Leu Asp Glu Gln Val Lys Ser Ala Ile Thr Glu Phe Gln Asn Val Gln
                325                 330                 335
Pro Thr Asn Glu Lys Met Thr Asp Leu Gln Asp Thr Lys Tyr Val Val
            340                 345                 350
Tyr Glu Ser Val Glu Asn Asn Glu Ser Met Met Asp Thr Phe Val Lys
        355                 360                 365
His Pro Ile Lys Thr Gly Met Leu Asn Gly Lys Lys Tyr Met Val Met
    370                 375                 380
Glu Thr Thr Asn Asp Asp Tyr Trp Lys Asp Phe Met Val Glu Gly Gln
385                 390                 395                 400
Arg Val Arg Thr Ile Ser Lys Asp Ala Lys Asn Asn Thr Arg Thr Ile
                405                 410                 415
Ile Phe Pro Tyr Val Glu Gly Lys Thr Leu Tyr Asp Ala Ile Val Lys
            420                 425                 430
```

```
Val His Val Lys Thr Ile Asp Tyr Asp Gly Gln Tyr His Val Arg Ile
        435                 440                 445
Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr Asp Lys Ser Asn Lys
    450                 455                 460
Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu Ala Thr Pro Ala Thr
465                 470                 475                 480
Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys Glu Ser Gln Lys Gln
                485                 490                 495
Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro Ser Val Glu Lys Glu
            500                 505                 510
Asn Asp Ala Ser Ser Glu Ser Gly Lys Asp Lys Thr Pro Ala Thr Lys
        515                 520                 525
Pro Thr Lys Gly Glu Val Glu Ser Ser Ser Thr Thr Pro Thr Lys Val
    530                 535                 540
Val Ser Thr Thr Gln Asn Val Ala Lys Pro Thr Thr Ala Ser Ser Lys
545                 550                 555                 560
Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly Ser Ser Glu Ala Lys
                565                 570                 575
Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
            580                 585                 590
His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln Glu Asn Lys Ala Lys
        595                 600                 605
Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro
    610                 615                 620
Leu Met Ala Leu Leu Ala Leu Ser Ser Ile Val Ala Phe Val Leu Pro
625                 630                 635                 640
Arg Lys Arg Lys Asn
                645
```

<210> 6
<211> 645
<212> PRT
<213> Artificial Sequence

<220>
<223> ORF0657n mutant

<400> 6

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Met Asn Lys Gln Gln Lys Glu Phe Lys Ser Phe Tyr Ser Ile Arg Lys
 1               5                  10                  15
Ser Ser Leu Gly Val Ala Ser Val Ala Ile Ser Thr Leu Leu Leu Leu
            20                  25                  30
Met Ser Asn Gly Glu Ala Gln Ala Ala Ala Glu Glu Thr Gly Gly Thr
        35                  40                  45
Asn Thr Glu Ala Gln Pro Lys Thr Glu Ala Val Ala Ser Pro Thr Thr
    50                  55                  60
Thr Ser Glu Lys Ala Pro Glu Thr Lys Pro Val Ala Asn Ala Val Ser
65                  70                  75                  80
Val Ser Asn Lys Glu Val Glu Ala Pro Thr Ser Glu Thr Lys Glu Ala
                85                  90                  95
Lys Glu Val Lys Glu Val Lys Ala Pro Lys Glu Thr Lys Glu Val Lys
            100                 105                 110
Pro Ala Ala Lys Ala Thr Asn Asn Thr Tyr Pro Ile Leu Asn Gln Glu
        115                 120                 125
```

```
Leu Arg Glu Ala Ile Lys Asn Pro Ala Ile Lys Asp Lys Asp His Ser
    130                 135                 140
Ala Pro Asn Trp Arg Pro Ile Asp Phe Glu Met Lys Lys Lys Asp Gly
145                 150                 155                 160
Thr Gln Gln Phe Tyr His Tyr Ala Ser Ser Val Glu Pro Ala Arg Val
                165                 170                 175
Ile Phe Thr Asp Ser Lys Pro Glu Ile Glu Leu Gly Leu Gln Ser Gly
            180                 185                 190
Gln Phe Trp Arg Lys Phe Glu Val Tyr Glu Gly Asp Lys Lys Leu Pro
        195                 200                 205
Ile Lys Leu Val Ser Tyr Asp Thr Val Lys Asp Tyr Ala Tyr Ile Arg
    210                 215                 220
Phe Ser Val Ser Asn Gly Thr Lys Ala Val Lys Ile Val Ser Ser Thr
225                 230                 235                 240
His Phe Asn Asn Lys Glu Glu Lys Tyr Asp Tyr Thr Leu Met Glu Phe
                245                 250                 255
Ala Gln Pro Ile Tyr Asn Ser Ala Asp Lys Phe Lys Thr Glu Glu Asp
            260                 265                 270
Tyr Lys Ala Glu Lys Leu Leu Ala Pro Tyr Lys Lys Ala Lys Thr Leu
        275                 280                 285
Glu Arg Gln Val Tyr Glu Leu Glu Lys Ile Gln Asp Lys Leu Pro Glu
    290                 295                 300
Lys Leu Lys Ala Glu Tyr Lys Lys Lys Leu Glu Asp Thr Lys Lys Ala
305                 310                 315                 320
Leu Asp Glu Gln Val Lys Ser Ala Ile Thr Glu Phe Gln Asn Val Gln
                325                 330                 335
Pro Thr Asn Glu Lys Met Thr Asp Leu Gln Asp Thr Lys Tyr Val Val
            340                 345                 350
Tyr Glu Ser Val Glu Asn Asn Glu Ser Met Met Asp Thr Phe Val Lys
        355                 360                 365
His Pro Ile Lys Thr Gly Met Leu Asn Gly Lys Lys Tyr Met Val Met
    370                 375                 380
Glu Thr Thr Asn Asp Asp Tyr Trp Lys Asp Phe Met Val Glu Gly Gln
385                 390                 395                 400
Arg Val Arg Thr Ile Ser Lys Asp Ala Lys Asn Asn Thr Arg Thr Ile
                405                 410                 415
Ile Phe Pro Tyr Val Glu Gly Lys Thr Leu Tyr Asp Ala Ile Val Lys
            420                 425                 430
Val His Val Lys Thr Ile Asp Tyr Asp Gly Gln Tyr His Val Arg Ile
        435                 440                 445
Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr Asp Lys Ser Asn Lys
    450                 455                 460
Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu Ala Thr Pro Ala Thr
465                 470                 475                 480
Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys Glu Ser Gln Lys Gln
                485                 490                 495
Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro Ser Val Glu Lys Glu
            500                 505                 510
Asn Asp Ala Ser Ser Glu Ser Gly Lys Asp Lys Thr Pro Ala Thr Lys
        515                 520                 525
Pro Thr Lys Gly Glu Val Glu Ser Ser Ser Thr Thr Pro Thr Lys Val
    530                 535                 540
Val Ser Thr Thr Gln Asn Val Ala Lys Pro Thr Thr Ala Ser Ser Lys
545                 550                 555                 560
```

```
Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly Ser Ser Glu Ala Lys
                565                 570                 575
Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
            580                 585                 590
His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln Glu Asn Lys Ala Lys
        595                 600                 605
Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro
    610                 615                 620
Leu Met Ala Leu Leu Ala Leu Ser Ser Ile Val Ala Phe Val Leu Pro
625                 630                 635                 640
Arg Lys Arg Lys Asn
                645
```

<210> 7
<211> 645
<212> PRT
<213> Artificial Sequence

<220>
<223> ORF0657n mutant

<400> 7

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Met Asn Lys Gln Gln Lys Glu Phe Lys Ser Phe Tyr Ser Ile Arg Lys
 1               5                  10                  15
Ser Ser Leu Gly Val Ala Ser Val Ala Ile Ser Thr Leu Leu Leu Leu
            20                  25                  30
Met Ser Asn Gly Glu Ala Gln Ala Ala Ala Glu Glu Thr Gly Gly Thr
        35                  40                  45
Asn Thr Glu Ala Gln Pro Lys Thr Glu Ala Val Ala Ser Pro Thr Thr
    50                  55                  60
Thr Ser Glu Lys Ala Pro Glu Thr Lys Pro Val Ala Asn Ala Val Ser
65                  70                  75                  80
Val Ser Asn Lys Glu Val Glu Ala Pro Thr Ser Glu Thr Lys Glu Ala
                85                  90                  95
Lys Glu Val Lys Glu Val Lys Ala Pro Lys Glu Thr Lys Glu Val Lys
            100                 105                 110
Pro Ala Ala Lys Ala Thr Asn Asn Thr Tyr Pro Ile Leu Asn Gln Glu
        115                 120                 125
Leu Arg Glu Ala Ile Lys Asn Pro Ala Ile Lys Asp Lys Asp His Ser
    130                 135                 140
Ala Pro Asn Trp Arg Pro Ile Asp Phe Glu Met Lys Lys Lys Asp Gly
145                 150                 155                 160
Thr Gln Gln Phe Tyr His Tyr Ala Ser Ser Val Glu Pro Ala Arg Val
                165                 170                 175
Ile Phe Thr Asp Ser Lys Pro Glu Ile Glu Leu Gly Leu Gln Ser Gly
            180                 185                 190
Gln Phe Trp Arg Lys Phe Glu Val Tyr Glu Gly Asp Lys Lys Leu Pro
        195                 200                 205
Ile Lys Leu Val Ser Tyr Asp Thr Val Lys Asp Tyr Ala Tyr Ile Arg
    210                 215                 220
Phe Ser Val Ser Asn Gly Thr Lys Glu Val Lys Ile Val Ser Ser Thr
225                 230                 235                 240
His Phe Asn Asn Lys Glu Glu Lys Tyr Asp Tyr Thr Leu Met Val Phe
                245                 250                 255
```

```
Ala Gln Pro Ile Tyr Asn Ser Ala Asp Lys Phe Lys Thr Glu Glu Asp
                260                 265                 270
Tyr Lys Ala Glu Lys Leu Leu Ala Pro Tyr Lys Lys Ala Lys Thr Leu
            275                 280                 285
Glu Arg Gln Val Tyr Glu Leu Asn Lys Ile Gln Asp Lys Leu Pro Glu
    290                 295                 300
Lys Leu Lys Ala Glu Tyr Lys Lys Lys Leu Glu Asp Thr Lys Lys Ala
305                 310                 315                 320
Leu Ala Glu Gln Val Lys Ser Ala Ile Thr Glu Phe Gln Asn Val Gln
                325                 330                 335
Pro Thr Asn Glu Lys Met Thr Asp Leu Gln Asp Thr Lys Tyr Val Val
            340                 345                 350
Tyr Glu Ser Val Glu Asn Asn Glu Ser Met Met Asp Thr Phe Val Lys
        355                 360                 365
His Pro Ile Lys Thr Gly Met Leu Asn Gly Lys Lys Tyr Met Val Met
    370                 375                 380
Glu Thr Thr Asn Asp Asp Tyr Trp Lys Asp Phe Met Val Glu Gly Gln
385                 390                 395                 400
Arg Val Arg Thr Ile Ser Lys Asp Ala Lys Asn Asn Thr Arg Thr Ile
                405                 410                 415
Ile Phe Pro Tyr Val Glu Gly Lys Thr Leu Tyr Asp Ala Ile Val Lys
            420                 425                 430
Val His Val Lys Thr Ile Asp Tyr Asp Gly Gln Tyr His Val Arg Ile
        435                 440                 445
Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr Asp Lys Ser Asn Lys
    450                 455                 460
Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu Ala Thr Pro Ala Thr
465                 470                 475                 480
Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys Glu Ser Gln Lys Gln
                485                 490                 495
Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro Ser Val Glu Lys Glu
            500                 505                 510
Asn Asp Ala Ser Ser Glu Ser Gly Lys Asp Lys Thr Pro Ala Thr Lys
        515                 520                 525
Pro Thr Lys Gly Glu Val Glu Ser Ser Ser Thr Thr Pro Thr Lys Val
    530                 535                 540
Val Ser Thr Thr Gln Asn Val Ala Lys Pro Thr Thr Ala Ser Ser Lys
545                 550                 555                 560
Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly Ser Ser Glu Ala Lys
                565                 570                 575
Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
            580                 585                 590
His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln Glu Asn Lys Ala Lys
        595                 600                 605
Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro
    610                 615                 620
Leu Met Ala Leu Leu Ala Leu Ser Ser Ile Val Ala Phe Val Leu Pro
625                 630                 635                 640
Arg Lys Arg Lys Asn
                645
```

<210> 8
<211> 645
<212> PRT
<213> Artificial Sequence

<220>
<223> ORF0657n mutant

<400> 8

Met	Asn	Lys	Gln	Gln	Lys	Glu	Phe	Lys	Ser	Phe	Tyr	Ser	Ile	Arg	Lys
1				5				10						15	
Ser	Ser	Leu	Gly	Val	Ala	Ser	Val	Ala	Ile	Ser	Thr	Leu	Leu	Leu	Leu
			20					25					30		
Met	Ser	Asn	Gly	Glu	Ala	Gln	Ala	Ala	Ala	Glu	Glu	Thr	Gly	Gly	Thr
		35					40					45			
Asn	Thr	Glu	Ala	Gln	Pro	Lys	Thr	Glu	Ala	Val	Ala	Ser	Pro	Thr	Thr
	50					55					60				
Thr	Ser	Glu	Lys	Ala	Pro	Glu	Thr	Lys	Pro	Val	Ala	Asn	Ala	Val	Ser
65					70					75					80
Val	Ser	Asn	Lys	Glu	Val	Glu	Ala	Pro	Thr	Ser	Glu	Thr	Lys	Glu	Ala
				85					90					95	
Lys	Glu	Val	Lys	Glu	Val	Lys	Ala	Pro	Lys	Glu	Thr	Lys	Glu	Val	Lys
			100					105					110		
Pro	Ala	Ala	Lys	Ala	Thr	Asn	Asn	Thr	Tyr	Pro	Ile	Leu	Asn	Gln	Glu
		115					120					125			
Leu	Arg	Glu	Ala	Ile	Lys	Asn	Pro	Ala	Ile	Lys	Asp	Lys	Asp	His	Ser
	130					135					140				
Ala	Pro	Asn	Trp	Arg	Pro	Ile	Asp	Phe	Glu	Met	Lys	Lys	Lys	Asp	Gly
145					150					155					160
Thr	Gln	Gln	Phe	Tyr	His	Tyr	Ala	Ser	Ser	Val	Glu	Pro	Ala	Arg	Val
				165					170					175	
Ile	Phe	Thr	Asp	Ser	Lys	Pro	Glu	Ile	Glu	Leu	Gly	Leu	Gln	Ser	Gly
			180					185					190		
Gln	Phe	Trp	Arg	Lys	Phe	Glu	Val	Tyr	Glu	Gly	Asp	Lys	Lys	Leu	Pro
		195					200					205			
Ile	Lys	Leu	Val	Ser	Tyr	Asp	Thr	Val	Lys	Asp	Tyr	Ala	Tyr	Ile	Arg
	210					215					220				
Phe	Ser	Val	Ser	Asn	Gly	Thr	Lys	Glu	Val	Lys	Ile	Val	Ser	Ser	Thr
225					230					235					240
His	Phe	Asn	Asn	Lys	Glu	Glu	Lys	Tyr	Asp	Tyr	Thr	Leu	Met	Glu	Phe
				245					250					255	
Ala	Gln	Pro	Ile	Tyr	Asn	Ser	Ala	Asp	Lys	Phe	Lys	Thr	Glu	Glu	Asp
			260					265					270		
Tyr	Lys	Ala	Glu	Lys	Leu	Leu	Ala	Pro	Tyr	Lys	Lys	Ala	Lys	Thr	Leu
		275					280					285			
Glu	Arg	Gln	Val	Tyr	Glu	Leu	Glu	Lys	Ile	Gln	Asp	Lys	Leu	Pro	Glu
	290					295					300				
Lys	Leu	Lys	Ala	Glu	Tyr	Lys	Lys	Lys	Leu	Glu	Asp	Thr	Lys	Lys	Ala
305					310					315					320
Leu	Ala	Glu	Gln	Val	Lys	Ser	Ala	Ile	Thr	Glu	Phe	Gln	Asn	Val	Gln
				325					330					335	
Pro	Thr	Asn	Glu	Lys	Met	Thr	Asp	Leu	Gln	Asp	Thr	Lys	Tyr	Val	Val
			340					345					350		
Tyr	Glu	Ser	Val	Glu	Asn	Asn	Glu	Ser	Met	Met	Asp	Thr	Phe	Val	Lys
		355					360					365			
His	Pro	Ile	Lys	Thr	Gly	Met	Leu	Asn	Gly	Lys	Lys	Tyr	Met	Val	Met
	370					375					380				
Glu	Thr	Thr	Asn	Asp	Asp	Tyr	Trp	Lys	Asp	Phe	Met	Val	Glu	Gly	Gln
385					390					395					400

```
Arg Val Arg Thr Ile Ser Lys Asp Ala Lys Asn Asn Thr Arg Thr Ile
                405                 410                 415
Ile Phe Pro Tyr Val Glu Gly Lys Thr Leu Tyr Asp Ala Ile Val Lys
            420                 425                 430
Val His Val Lys Thr Ile Asp Tyr Asp Gly Gln Tyr His Val Arg Ile
        435                 440                 445
Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr Asp Lys Ser Asn Lys
    450                 455                 460
Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu Ala Thr Pro Ala Thr
465                 470                 475                 480
Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys Glu Ser Gln Lys Gln
                485                 490                 495
Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro Ser Val Glu Lys Glu
            500                 505                 510
Asn Asp Ala Ser Ser Glu Ser Gly Lys Asp Lys Thr Pro Ala Thr Lys
        515                 520                 525
Pro Thr Lys Gly Glu Val Glu Ser Ser Ser Thr Thr Pro Thr Lys Val
    530                 535                 540
Val Ser Thr Thr Gln Asn Val Ala Lys Pro Thr Thr Ala Ser Ser Lys
545                 550                 555                 560
Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly Ser Ser Glu Ala Lys
                565                 570                 575
Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
            580                 585                 590
His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln Glu Asn Lys Ala Lys
        595                 600                 605
Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro
    610                 615                 620
Leu Met Ala Leu Leu Ala Leu Ser Ser Ile Val Ala Phe Val Leu Pro
625                 630                 635                 640
Arg Lys Arg Lys Asn
                645
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<210> 9
<211> 645
<212> PRT
<213> Artificial Sequence

<220>
<223> ORF0657n mutant

<400> 9

```
Met Asn Lys Gln Gln Lys Glu Phe Lys Ser Phe Tyr Ser Ile Arg Lys
 1                5                  10                  15
Ser Ser Leu Gly Val Ala Ser Val Ala Ile Ser Thr Leu Leu Leu Leu
            20                  25                  30
Met Ser Asn Gly Glu Ala Gln Ala Ala Ala Glu Glu Thr Gly Gly Thr
        35                  40                  45
Asn Thr Glu Ala Gln Pro Lys Thr Glu Ala Val Ala Ser Pro Thr Thr
    50                  55                  60
Thr Ser Glu Lys Ala Pro Glu Thr Lys Pro Val Ala Asn Ala Val Ser
65                  70                  75                  80
Val Ser Asn Lys Glu Val Glu Ala Pro Thr Ser Glu Thr Lys Glu Ala
                85                  90                  95
```

```
Lys Glu Val Lys Glu Val Lys Ala Pro Lys Glu Thr Lys Glu Val Lys
                100                 105                 110
Pro Ala Ala Lys Ala Thr Asn Asn Thr Tyr Pro Ile Leu Asn Gln Glu
            115                 120                 125
Leu Arg Glu Ala Ile Lys Asn Pro Ala Ile Lys Asp Lys Asp His Ser
    130                 135                 140
Ala Pro Asn Trp Arg Pro Ile Asp Phe Glu Met Lys Lys Lys Asp Gly
145                 150                 155                 160
Thr Gln Gln Phe Tyr His Tyr Ala Ser Ser Val Glu Pro Ala Arg Val
                165                 170                 175
Ile Phe Thr Lys Ser Lys Pro Glu Ile Glu Leu Gly Leu Gln Ser Gly
            180                 185                 190
Gln Phe Trp Arg Lys Phe Glu Val Tyr Glu Gly Asp Lys Lys Leu Pro
        195                 200                 205
Ile Lys Leu Val Ser Tyr Asp Thr Asp Lys Asp Tyr Ala Tyr Ile Arg
    210                 215                 220
Phe Ser Val Ser Asn Gly Thr Lys Glu Val Lys Ile Val Ser Ser Thr
225                 230                 235                 240
His Phe Asn Asn Lys Glu Glu Lys Tyr Asp Tyr Thr Leu Met Glu Phe
                245                 250                 255
Ala Gln Pro Ile Tyr Asn Ser Ala Asp Lys Phe Lys Thr Glu Glu Asp
            260                 265                 270
Tyr Lys Ala Glu Lys Leu Leu Ala Pro Tyr Lys Lys Ala Lys Thr Leu
        275                 280                 285
Glu Arg Gln Val Tyr Glu Leu Glu Lys Ile Gln Asp Lys Leu Pro Glu
    290                 295                 300
Lys Leu Lys Ala Glu Tyr Lys Lys Lys Leu Glu Asp Thr Lys Lys Ala
305                 310                 315                 320
Leu Ala Glu Gln Val Lys Ser Ala Ile Thr Glu Phe Gln Asn Val Gln
                325                 330                 335
Pro Thr Asn Glu Lys Met Thr Asp Leu Gln Asp Thr Lys Tyr Val Val
            340                 345                 350
Tyr Glu Ser Val Glu Asn Asn Glu Ser Met Met Asp Thr Phe Val Lys
        355                 360                 365
His Pro Ile Lys Thr Gly Met Leu Asn Gly Lys Lys Tyr Met Val Met
    370                 375                 380
Glu Thr Thr Asn Asp Asp Tyr Trp Lys Asp Phe Met Val Glu Gly Gln
385                 390                 395                 400
Arg Val Arg Thr Ile Ser Lys Asp Ala Lys Asn Asn Thr Arg Thr Ile
                405                 410                 415
Ile Phe Pro Tyr Val Glu Gly Lys Thr Leu Tyr Asp Ala Ile Val Lys
            420                 425                 430
Val His Val Lys Thr Ile Asp Tyr Asp Gly Gln Tyr His Val Arg Ile
        435                 440                 445
Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr Asp Lys Ser Asn Lys
    450                 455                 460
Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu Ala Thr Pro Ala Thr
465                 470                 475                 480
Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys Glu Ser Gln Lys Gln
                485                 490                 495
Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro Ser Val Glu Lys Glu
            500                 505                 510
Asn Asp Ala Ser Ser Glu Ser Gly Lys Asp Lys Thr Pro Ala Thr Lys
        515                 520                 525
```

```
Pro Thr Lys Gly Glu Val Glu Ser Ser Ser Thr Thr Pro Thr Lys Val
    530                 535                 540
Val Ser Thr Thr Gln Asn Val Ala Lys Pro Thr Thr Ala Ser Ser Lys
545                 550                 555                 560
Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly Ser Ser Glu Ala Lys
                565                 570                 575
Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
            580                 585                 590
His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln Glu Asn Lys Ala Lys
        595                 600                 605
Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro
    610                 615                 620
Leu Met Ala Leu Leu Ala Leu Ser Ser Ile Val Ala Phe Val Leu Pro
625                 630                 635                 640
Arg Lys Arg Lys Asn
                645
```

<210> 10
<211> 645
<212> PRT
<213> Artificial Sequence

<220>
<223> ORF0657n mutant

<400> 10

```
Met Asn Lys Gln Gln Lys Glu Phe Lys Ser Phe Tyr Ser Ile Arg Lys
 1               5                  10                  15
Ser Ser Leu Gly Val Ala Ser Val Ala Ile Ser Thr Leu Leu Leu Leu
            20                  25                  30
Met Ser Asn Gly Glu Ala Gln Ala Ala Ala Glu Glu Thr Gly Gly Thr
        35                  40                  45
Asn Thr Glu Ala Gln Pro Lys Thr Glu Ala Val Ala Ser Pro Thr Thr
    50                  55                  60
Thr Ser Glu Lys Ala Pro Glu Thr Lys Pro Val Ala Asn Ala Val Ser
65                  70                  75                  80
Val Ser Asn Lys Glu Val Glu Ala Pro Thr Ser Glu Thr Lys Glu Ala
                85                  90                  95
Lys Glu Val Lys Glu Val Lys Ala Pro Lys Glu Thr Lys Glu Val Lys
            100                 105                 110
Pro Ala Ala Lys Ala Thr Asn Asn Thr Tyr Pro Ile Leu Asn Gln Glu
        115                 120                 125
Leu Arg Glu Ala Ile Lys Asn Pro Ala Ile Lys Asp Lys Asp His Ser
    130                 135                 140
Ala Pro Asn Trp Arg Pro Ile Asp Phe Glu Met Lys Lys Lys Asp Gly
145                 150                 155                 160
Thr Gln Gln Phe Tyr His Tyr Ala Ser Ser Val Glu Pro Ala Arg Val
                165                 170                 175
Ile Phe Thr Lys Ser Lys Pro Glu Ile Glu Leu Gly Leu Gln Ser Gly
            180                 185                 190
Ser Thr Trp Arg Lys Phe Glu Val Tyr Glu Gly Asp Lys Lys Leu Pro
        195                 200                 205
Ile Lys Leu Val Ser Tyr Asp Thr Asp Lys Asp Tyr Ala Tyr Ile Arg
    210                 215                 220
```

```
Phe Ser Val Ser Asn Gly Thr Lys Glu Val Lys Ile Val Ser Ser Thr
225                 230                 235                 240
His Phe Asn Asn Lys Glu Glu Lys Tyr Asp Tyr Thr Leu Met Val Phe
                245                 250                 255
Ala Gln Pro Ile Tyr Asn Ser Ala Asp Lys Phe Lys Thr Glu Glu Asp
            260                 265                 270
Tyr Lys Ala Glu Lys Leu Leu Ala Pro Tyr Lys Lys Ala Lys Thr Leu
        275                 280                 285
Glu Arg Gln Val Tyr Glu Leu Glu Lys Ile Gln Asp Lys Leu Pro Glu
    290                 295                 300
Lys Leu Lys Ala Glu Tyr Lys Lys Lys Leu Glu Asp Thr Lys Lys Ala
305                 310                 315                 320
Leu Ala Glu Gln Val Lys Ser Ala Ile Thr Glu Phe Gln Asn Val Gln
                325                 330                 335
Pro Thr Asn Glu Lys Met Thr Asp Leu Gln Asp Thr Lys Tyr Val Val
            340                 345                 350
Tyr Glu Ser Val Glu Asn Asn Glu Ser Met Met Asp Thr Phe Val Lys
        355                 360                 365
His Pro Ile Lys Thr Gly Met Leu Asn Gly Lys Lys Tyr Met Val Met
    370                 375                 380
Glu Thr Thr Asn Asp Asp Tyr Trp Lys Asp Phe Met Val Glu Gly Gln
385                 390                 395                 400
Arg Val Arg Thr Ile Ser Lys Asp Ala Lys Asn Asn Thr Arg Thr Ile
                405                 410                 415
Ile Phe Pro Tyr Val Glu Gly Lys Thr Leu Tyr Asp Ala Ile Val Lys
            420                 425                 430
Val His Val Lys Thr Ile Asp Tyr Asp Gly Gln Tyr His Val Arg Ile
        435                 440                 445
Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr Asp Lys Ser Asn Lys
    450                 455                 460
Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu Ala Thr Pro Ala Thr
465                 470                 475                 480
Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys Glu Ser Gln Lys Gln
                485                 490                 495
Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro Ser Val Glu Lys Glu
            500                 505                 510
Asn Asp Ala Ser Ser Glu Ser Gly Lys Asp Lys Thr Pro Ala Thr Lys
        515                 520                 525
Pro Thr Lys Gly Glu Val Glu Ser Ser Ser Thr Thr Pro Thr Lys Val
    530                 535                 540
Val Ser Thr Thr Gln Asn Val Ala Lys Pro Thr Thr Ala Ser Ser Lys
545                 550                 555                 560
Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly Ser Ser Glu Ala Lys
                565                 570                 575
Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
            580                 585                 590
His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln Glu Asn Lys Ala Lys
        595                 600                 605
Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro
    610                 615                 620
Leu Met Ala Leu Leu Ala Leu Ser Ser Ile Val Ala Phe Val Leu Pro
625                 630                 635                 640
Arg Lys Arg Lys Asn
                645
```

<210> 11
<211> 645
<212> PRT
<213> Artificial Sequence

<220>
<223> ORF0657n mutant

<400> 11

Met	Asn	Lys	Gln	Gln	Lys	Glu	Phe	Lys	Ser	Phe	Tyr	Ser	Ile	Arg	Lys
1				5				10					15		
Ser	Ser	Leu	Gly	Val	Ala	Ser	Val	Ala	Ile	Ser	Thr	Leu	Leu	Leu	Leu
			20					25					30		
Met	Ser	Asn	Gly	Glu	Ala	Gln	Ala	Ala	Ala	Glu	Glu	Thr	Gly	Gly	Thr
		35					40					45			
Asn	Thr	Glu	Ala	Gln	Pro	Lys	Thr	Glu	Ala	Val	Ala	Ser	Pro	Thr	Thr
	50					55					60				
Thr	Ser	Glu	Lys	Ala	Pro	Glu	Thr	Lys	Pro	Val	Ala	Asn	Ala	Val	Ser
65					70					75					80
Val	Ser	Asn	Lys	Glu	Val	Glu	Ala	Pro	Thr	Ser	Glu	Thr	Lys	Glu	Ala
				85					90					95	
Lys	Glu	Val	Lys	Glu	Val	Lys	Ala	Pro	Lys	Glu	Thr	Lys	Glu	Val	Lys
			100					105					110		
Pro	Ala	Ala	Lys	Ala	Thr	Asn	Asn	Thr	Tyr	Pro	Ile	Leu	Asn	Gln	Glu
		115					120					125			
Leu	Arg	Glu	Ala	Ile	Lys	Asn	Pro	Ala	Ile	Lys	Asp	Lys	Asp	His	Ser
	130					135					140				
Ala	Pro	Asn	Trp	Arg	Pro	Ile	Asp	Phe	Glu	Met	Lys	Asn	Asp	Lys	Gly
145					150					155					160
Thr	Gln	Gln	Phe	Tyr	His	Tyr	Ala	Ser	Ser	Val	Glu	Pro	Ala	Arg	Val
				165					170					175	
Ile	Phe	Thr	Lys	Ser	Lys	Pro	Ile	Ile	Glu	Leu	Gly	Leu	Gln	Ser	Gly
			180					185					190		
Gln	Phe	Trp	Arg	Lys	Phe	Glu	Val	Tyr	Glu	Gly	Asp	Lys	Lys	Leu	Pro
		195					200					205			
Ile	Lys	Leu	Val	Ser	Tyr	Asp	Thr	Asp	Lys	Asp	Tyr	Ala	Tyr	Ile	Arg
	210					215					220				
Phe	Ser	Val	Ser	Asn	Gly	Thr	Lys	Glu	Val	Lys	Ile	Val	Ser	Ser	Thr
225					230					235					240
His	Phe	Asn	Asn	Lys	Glu	Glu	Lys	Tyr	Asp	Tyr	Thr	Leu	Met	Val	Phe
				245					250					255	
Ala	Gln	Pro	Ile	Tyr	Asn	Ser	Ala	Asp	Lys	Phe	Lys	Thr	Glu	Glu	Asp
			260					265					270		
Tyr	Lys	Ala	Glu	Lys	Leu	Leu	Ala	Pro	Tyr	Lys	Lys	Ala	Lys	Thr	Leu
		275					280					285			
Glu	Arg	Gln	Val	Tyr	Glu	Leu	Glu	Lys	Ile	Gln	Asp	Lys	Leu	Pro	Glu
	290					295					300				
Lys	Leu	Lys	Ala	Glu	Tyr	Lys	Lys	Lys	Leu	Glu	Asp	Thr	Lys	Lys	Ala
305					310					315					320
Leu	Ala	Glu	Gln	Val	Lys	Ser	Ala	Ile	Thr	Glu	Phe	Gln	Asn	Val	Gln
				325					330					335	
Pro	Thr	Asn	Glu	Lys	Met	Thr	Asp	Leu	Gln	Asp	Thr	Lys	Tyr	Val	Val
			340					345					350		
Tyr	Glu	Ser	Val	Glu	Asn	Asn	Glu	Ser	Met	Met	Asp	Thr	Phe	Val	Lys
		355					360					365			

```
His Pro Ile Lys Thr Gly Met Leu Asn Gly Lys Lys Tyr Met Val Met
    370                 375                 380
Glu Thr Thr Asn Asp Asp Tyr Trp Lys Asp Phe Met Val Glu Gly Gln
385                 390                 395                 400
Arg Val Arg Thr Ile Ser Lys Asp Ala Lys Asn Asn Thr Arg Thr Ile
                405                 410                 415
Ile Phe Pro Tyr Val Glu Gly Lys Thr Leu Tyr Asp Ala Ile Val Lys
            420                 425                 430
Val His Val Lys Thr Ile Asp Tyr Asp Gly Gln Tyr His Val Arg Ile
        435                 440                 445
Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr Asp Lys Ser Asn Lys
    450                 455                 460
Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu Ala Thr Pro Ala Thr
465                 470                 475                 480
Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys Glu Ser Gln Lys Gln
                485                 490                 495
Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro Ser Val Glu Lys Glu
            500                 505                 510
Asn Asp Ala Ser Ser Glu Ser Gly Lys Asp Lys Thr Pro Ala Thr Lys
        515                 520                 525
Pro Thr Lys Gly Glu Val Glu Ser Ser Ser Thr Thr Pro Thr Lys Val
    530                 535                 540
Val Ser Thr Thr Gln Asn Val Ala Lys Pro Thr Thr Ala Ser Ser Lys
545                 550                 555                 560
Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly Ser Ser Glu Ala Lys
                565                 570                 575
Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
            580                 585                 590
His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln Glu Asn Lys Ala Lys
        595                 600                 605
Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro
    610                 615                 620
Leu Met Ala Leu Leu Ala Leu Ser Ser Ile Val Ala Phe Val Leu Pro
625                 630                 635                 640
Arg Lys Arg Lys Asn
                645
```

<210> 12
<211> 645
<212> PRT
<213> Artificial Sequence

<220>
<223> ORF0657n mutant

<400> 12

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Met Asn Lys Gln Gln Lys Glu Phe Lys Ser Phe Tyr Ser Ile Arg Lys
 1               5                  10                  15
Ser Ser Leu Gly Val Ala Ser Val Ala Ile Ser Thr Leu Leu Leu Leu
            20                  25                  30
Met Ser Asn Gly Glu Ala Gln Ala Ala Ala Glu Glu Thr Gly Gly Thr
        35                  40                  45
Asn Thr Glu Ala Gln Pro Lys Thr Glu Ala Val Ala Ser Pro Thr Thr
    50                  55                  60
```

```
Thr Ser Glu Lys Ala Pro Glu Thr Lys Pro Val Ala Asn Ala Val Ser
65                  70                  75                  80
Val Ser Asn Lys Glu Val Glu Ala Pro Thr Ser Glu Thr Lys Glu Ala
                85                  90                  95
Lys Glu Val Lys Glu Val Lys Ala Pro Lys Glu Thr Lys Glu Val Lys
            100                 105                 110
Pro Ala Ala Lys Ala Thr Asn Asn Thr Tyr Pro Ile Leu Asn Gln Glu
        115                 120                 125
Leu Arg Glu Ala Ile Lys Asn Pro Ala Ile Lys Asp Lys Asp His Ser
    130                 135                 140
Ala Pro Asn Trp Arg Pro Ile Asp Phe Glu Met Lys Asn Asp Lys Gly
145                 150                 155                 160
Thr Gln Gln Phe Tyr His Tyr Ala Ser Ser Val Glu Pro Ala Arg Val
                165                 170                 175
Ile Phe Thr Lys Ser Lys Pro Ile Ile Glu Leu Gly Leu Gln Ser Gly
            180                 185                 190
Gln Phe Trp Arg Lys Phe Glu Val Tyr Glu Gly Asp Lys Lys Leu Pro
        195                 200                 205
Ile Lys Leu Val Ser Tyr Asp Thr Asp Lys Asp Tyr Ala Tyr Ile Arg
    210                 215                 220
Phe Ser Val Ser Asn Gly Thr Lys Glu Val Lys Ile Val Ser Ser Thr
225                 230                 235                 240
His Phe Asn Asn Lys Glu Glu Lys Tyr Asp Tyr Thr Leu Met Val Phe
                245                 250                 255
Ala Gln Pro Ile Tyr Asn Ser Ala Asp Lys Phe Lys Thr Glu Glu Asp
            260                 265                 270
Tyr Lys Ala Glu Lys Leu Leu Ala Pro Tyr Lys Lys Ala Lys Thr Leu
        275                 280                 285
Glu Arg Gln Val Tyr Glu Leu Glu Lys Ile Gln Asp Lys Leu Pro Glu
    290                 295                 300
Lys Leu Lys Ala Glu Tyr Lys Lys Lys Leu Glu Gln Thr Lys Lys Ala
305                 310                 315                 320
Leu Ala Glu Gln Val Lys Ser Ala Ile Thr Glu Phe Gln Asn Val Gln
                325                 330                 335
Pro Thr Asn Glu Lys Met Thr Asp Leu Gln Asp Ala His Tyr Val Val
            340                 345                 350
Tyr Glu Ser Val Glu Asn Ser Glu Ser Met Met Asp Thr Phe Val Lys
        355                 360                 365
His Pro Ile Lys Thr Gly Met Leu Asn Gly Lys Lys Tyr Met Val Met
    370                 375                 380
Glu Thr Thr Asn Asp Asp Tyr Trp Lys Asp Phe Met Val Glu Gly Gln
385                 390                 395                 400
Arg Val Arg Thr Ile Ser Lys Asp Ala Lys Asn Asn Thr Arg Thr Ile
                405                 410                 415
Ile Phe Pro Tyr Val Glu Gly Lys Thr Leu Tyr Asp Ala Ile Val Lys
            420                 425                 430
Val His Val Lys Thr Ile Asp Tyr Asp Gly Gln Tyr His Val Arg Ile
        435                 440                 445
Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr Asp Lys Ser Asn Lys
    450                 455                 460
Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu Ala Thr Pro Ala Thr
465                 470                 475                 480
Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys Glu Ser Gln Lys Gln
                485                 490                 495
```

```
Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro Ser Val Glu Lys Glu
                500                 505                 510
Asn Asp Ala Ser Ser Glu Ser Gly Lys Asp Lys Thr Pro Ala Thr Lys
        515                 520                 525
Pro Thr Lys Gly Glu Val Glu Ser Ser Ser Thr Thr Pro Thr Lys Val
    530                 535                 540
Val Ser Thr Thr Gln Asn Val Ala Lys Pro Thr Thr Ala Ser Ser Lys
545                 550                 555                 560
Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly Ser Ser Glu Ala Lys
                565                 570                 575
Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
            580                 585                 590
His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln Glu Asn Lys Ala Lys
        595                 600                 605
Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro
    610                 615                 620
Leu Met Ala Leu Leu Ala Leu Ser Ser Ile Val Ala Phe Val Leu Pro
625                 630                 635                 640
Arg Lys Arg Lys Asn
                645
```

<210> 13
<211> 645
<212> PRT
<213> Artificial Sequence

<220>
<223> ORF0657n mutant

<400> 13

```
Met Asn Lys Gln Gln Lys Glu Phe Lys Ser Phe Tyr Ser Ile Arg Lys
 1                5                  10                  15
Ser Ser Leu Gly Val Ala Ser Val Ala Ile Ser Thr Leu Leu Leu Leu
            20                  25                  30
Met Ser Asn Gly Glu Ala Gln Ala Ala Ala Glu Glu Thr Gly Gly Thr
        35                  40                  45
Asn Thr Glu Ala Gln Pro Lys Thr Glu Ala Val Ala Ser Pro Thr Thr
    50                  55                  60
Thr Ser Glu Lys Ala Pro Glu Thr Lys Pro Val Ala Asn Ala Val Ser
65                  70                  75                  80
Val Ser Asn Lys Glu Val Glu Ala Pro Thr Ser Glu Thr Lys Glu Ala
                85                  90                  95
Lys Glu Val Lys Glu Val Lys Ala Pro Lys Glu Thr Lys Glu Val Lys
            100                 105                 110
Pro Ala Ala Lys Ala Thr Asn Asn Thr Tyr Pro Ile Leu Asn Gln Glu
        115                 120                 125
Leu Arg Glu Ala Ile Lys Asn Pro Ala Ile Lys Asp Lys Asp His Ser
    130                 135                 140
Ala Pro Asn Trp Arg Pro Ile Asp Phe Glu Met Lys Asn Asp Lys Gly
145                 150                 155                 160
Thr Gln Gln Phe Tyr His Tyr Ala Ser Ser Val Glu Pro Ala Arg Val
                165                 170                 175
Ile Phe Thr Lys Ser Lys Pro Ile Ile Glu Leu Gly Leu Gln Ser Gly
            180                 185                 190
```

```
Gln Phe Trp Arg Lys Phe Glu Val Tyr Glu Gly Asp Lys Lys Leu Pro
        195                 200                 205
Ile Lys Leu Val Ser Tyr Asp Thr Asp Lys Asp Tyr Ala Tyr Ile Arg
    210                 215                 220
Phe Ser Val Ser Asn Gly Thr Lys Glu Val Lys Ile Val Ser Ser Thr
225                 230                 235                 240
His Phe Asn Asn Lys Glu Glu Lys Tyr Asp Tyr Thr Leu Met Val Phe
                245                 250                 255
Ala Gln Pro Ile Tyr Asn Ser Ala Asp Lys Phe Lys Thr Glu Glu Asp
            260                 265                 270
Tyr Lys Ala Glu Lys Leu Leu Ala Pro Tyr Lys Lys Ala Lys Thr Leu
        275                 280                 285
Glu Arg Gln Val Tyr Glu Leu Glu Lys Ile Gln Asp Lys Leu Pro Glu
    290                 295                 300
Lys Leu Lys Ala Glu Tyr Lys Lys Lys Leu Glu Gln Thr Lys Lys Ala
305                 310                 315                 320
Leu Ala Glu Gln Val Lys Ser Ala Ile Thr Glu Phe Gln Asn Val Gln
                325                 330                 335
Pro Thr Asn Glu Lys Met Thr Asp Leu Gln Asp Ala His Tyr Val Val
            340                 345                 350
Tyr Glu Ser Val Glu Asn Ser Glu Ser Met Met Asp Thr Phe Val Lys
        355                 360                 365
His Pro Ile Lys Thr Gly Met Leu Asn Gly Lys Lys Tyr Met Val Met
    370                 375                 380
Glu Thr Thr Asn Asp Asp Tyr Trp Lys Asp Phe Met Val Glu Gly Lys
385                 390                 395                 400
Arg Val Arg Thr Ile Ser Lys Asp Ala Lys Asn Asn Thr Arg Thr Ile
                405                 410                 415
Ile Phe Pro Tyr Val Glu Gly Lys Ala Leu Tyr Asp Ala Ile Val Lys
            420                 425                 430
Val His Val Lys Thr Ile Asp Tyr Asp Gly Gln Tyr His Val Arg Ile
        435                 440                 445
Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr Asp Lys Ser Asn Lys
    450                 455                 460
Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu Ala Thr Pro Ala Thr
465                 470                 475                 480
Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys Glu Ser Gln Lys Gln
                485                 490                 495
Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro Ser Val Glu Lys Glu
            500                 505                 510
Asn Asp Ala Ser Ser Glu Ser Gly Lys Asp Lys Thr Pro Ala Thr Lys
        515                 520                 525
Pro Thr Lys Gly Glu Val Glu Ser Ser Ser Thr Thr Pro Thr Lys Val
    530                 535                 540
Val Ser Thr Thr Gln Asn Val Ala Lys Pro Thr Thr Ala Ser Ser Lys
545                 550                 555                 560
Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly Ser Ser Glu Ala Lys
                565                 570                 575
Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
            580                 585                 590
His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln Glu Asn Lys Ala Lys
        595                 600                 605
Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro
    610                 615                 620
```

Leu Met Ala Leu Leu Ala Leu Ser Ser Ile Val Ala Phe Val Leu Pro
625 630 635 640
Arg Lys Arg Lys Asn
645

<210> 14
<211> 645
<212> PRT
<213> Artificial Sequence

<220>
<223> ORF0657n mutant

<400> 14
Met Asn Lys Gln Gln Lys Glu Phe Lys Ser Phe Tyr Ser Ile Arg Lys
1 5 10 15
Ser Ser Leu Gly Val Ala Ser Val Ala Ile Ser Thr Leu Leu Leu Leu
20 25 30
Met Ser Asn Gly Glu Ala Gln Ala Ala Ala Glu Glu Thr Gly Gly Thr
35 40 45
Asn Thr Glu Ala Gln Pro Lys Thr Glu Ala Val Ala Ser Pro Thr Thr
50 55 60
Thr Ser Glu Lys Ala Pro Glu Thr Lys Pro Val Ala Asn Ala Val Ser
65 70 75 80
Val Ser Asn Lys Glu Val Glu Ala Pro Thr Ser Glu Thr Lys Glu Ala
85 90 95
Lys Glu Val Lys Glu Val Lys Ala Pro Lys Glu Thr Lys Glu Val Lys
100 105 110
Pro Ala Ala Lys Ala Thr Asn Asn Thr Tyr Pro Ile Leu Asn Gln Glu
115 120 125
Leu Arg Glu Ala Ile Lys Asn Pro Ala Ile Lys Asp Lys Glu His Ser
130 135 140
Ala Pro Asn Ser Arg Pro Ile Asp Phe Glu Met Lys Lys Lys Asp Gly
145 150 155 160
Thr Gln Gln Phe Tyr His Tyr Ala Ser Ser Val Lys Pro Ala Arg Val
165 170 175
Ile Phe Thr Asp Ser Lys Pro Glu Ile Glu Leu Gly Leu Gln Ser Gly
180 185 190
Gln Phe Trp Arg Lys Phe Glu Val Tyr Glu Gly Asp Lys Lys Leu Pro
195 200 205
Val Lys Leu Val Ser Tyr Asp Thr Val Lys Asp Tyr Ala Tyr Ile Arg
210 215 220
Phe Ser Val Ser Asn Gly Thr Lys Ala Val Lys Ile Val Ser Ser Thr
225 230 235 240
His Phe Asn Asn Lys Glu Glu Lys Tyr Asp Tyr Thr Leu Met Glu Phe
245 250 255
Ala Gln Pro Ile Tyr Asn Ser Ala Asp Lys Phe Lys Thr Glu Glu Asp
260 265 270
Tyr Lys Ala Glu Lys Leu Leu Ala Pro Tyr Lys Lys Ala Lys Thr Leu
275 280 285
Glu Arg Gln Val Tyr Glu Leu Asn Lys Leu Gln Glu Lys Leu Pro Glu
290 295 300
Lys Leu Lys Ala Glu Tyr Lys Lys Lys Leu Glu Asp Thr Lys Lys Ala
305 310 315 320

```
Leu Asp Glu Gln Val Lys Ser Ala Val Thr Glu Phe Gln Asn Val Gln
                325                 330                 335
Pro Thr Asn Asp Lys Met Thr Asp Leu Gln Asp Thr Lys Tyr Val Val
            340                 345                 350
Tyr Glu Ser Val Glu Asn Asn Glu Ser Met Met Asp Thr Phe Val Lys
        355                 360                 365
His Pro Ile Lys Thr Gly Met Leu Asn Gly Lys Lys Tyr Met Val Met
    370                 375                 380
Glu Thr Thr Asn Asp Asp Tyr Trp Lys Asp Phe Met Val Glu Gly Gln
385                 390                 395                 400
Ser Val Arg Thr Ile Ser Lys Asp Ala Lys Asn Asn Thr Arg Thr Ile
                405                 410                 415
Ile Phe Pro Tyr Ile Glu Gly Lys Thr Leu Tyr Asp Ala Ile Val Lys
            420                 425                 430
Val His Val Lys Thr Ile Asp Tyr Asp Gly Gln Tyr His Val Arg Ile
        435                 440                 445
Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr Asp Lys Ser Asn Lys
    450                 455                 460
Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu Ala Thr Pro Ala Thr
465                 470                 475                 480
Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys Glu Ser Gln Lys Gln
                485                 490                 495
Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro Ser Val Glu Lys Glu
            500                 505                 510
Asn Asp Ala Ser Ser Glu Ser Gly Lys Asp Lys Thr Pro Ala Thr Lys
        515                 520                 525
Pro Thr Lys Gly Glu Val Glu Ser Ser Ser Thr Thr Pro Thr Lys Val
    530                 535                 540
Val Ser Thr Thr Gln Asn Val Ala Lys Pro Thr Thr Ala Ser Ser Lys
545                 550                 555                 560
Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly Ser Ser Glu Ala Lys
                565                 570                 575
Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
            580                 585                 590
His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln Glu Asn Lys Ala Lys
        595                 600                 605
Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro
    610                 615                 620
Leu Met Ala Leu Leu Ala Leu Ser Ser Ile Val Ala Phe Val Leu Pro
625                 630                 635                 640
Arg Lys Arg Lys Asn
                645
```

<210> 15
<211> 645
<212> PRT
<213> Artificial Sequence

<220>
<223> ORF0657n mutant

<400> 15

```
Met Asn Lys Gln Gln Lys Glu Phe Lys Ser Phe Tyr Ser Ile Arg Lys
 1                 5                  10                  15
```

```
Ser Ser Leu Gly Val Ala Ser Val Ala Ile Ser Thr Leu Leu Leu Leu
                20                  25                  30
Met Ser Asn Gly Glu Ala Gln Ala Ala Ala Glu Glu Thr Gly Gly Thr
            35                  40                  45
Asn Thr Glu Ala Gln Pro Lys Thr Glu Ala Val Ala Ser Pro Thr Thr
        50                  55                  60
Thr Ser Glu Lys Ala Pro Glu Thr Lys Pro Val Ala Asn Ala Val Ser
65                  70                  75                  80
Val Ser Asn Lys Glu Val Glu Ala Pro Thr Ser Glu Thr Lys Glu Ala
                85                  90                  95
Lys Glu Val Lys Glu Val Lys Ala Pro Lys Glu Thr Lys Glu Val Lys
            100                 105                 110
Pro Ala Ala Lys Ala Thr Asn Asn Thr Tyr Pro Ile Leu Asn Gln Glu
        115                 120                 125
Leu Arg Asp Ala Ile Lys Asn Pro Ala Ile Lys Asp Lys Glu His Ser
    130                 135                 140
Ala Pro Asn Ser Arg Pro Ile Asp Phe Glu Met Lys Lys Lys Asp Gly
145                 150                 155                 160
Thr Gln Gln Phe Tyr His Tyr Ala Ser Thr Val Lys Pro Ala Arg Val
                165                 170                 175
Ile Phe Thr Asp Thr Lys Pro Glu Ile Glu Leu Gly Leu Gln Ser Gly
            180                 185                 190
Gln Phe Trp Arg Lys Phe Glu Val Tyr Glu Gly Asp Lys Lys Leu Pro
        195                 200                 205
Val Lys Leu Val Ser Tyr Asp Ser Val Lys Asp Tyr Ala Tyr Ile Arg
    210                 215                 220
Phe Ser Val Ser Asn Gly Thr Arg Ala Val Lys Ile Val Ser Ser Thr
225                 230                 235                 240
His Tyr Asn Asn Lys Glu Glu Lys Tyr Asp Tyr Thr Leu Met Glu Phe
                245                 250                 255
Ala Gln Pro Ile Tyr Asn Ser Ala Asp Lys Tyr Lys Thr Glu Glu Asp
            260                 265                 270
Tyr Lys Ala Glu Lys Leu Leu Ala Pro Tyr Lys Lys Ala Lys Thr Leu
        275                 280                 285
Glu Arg Gln Val Tyr Glu Leu Asn Lys Leu Gln Asp Lys Leu Pro Glu
    290                 295                 300
Lys Leu Lys Ala Glu Tyr Lys Lys Lys Leu Asp Asp Thr Lys Lys Ala
305                 310                 315                 320
Leu Asp Asp Gln Val Lys Ser Ala Val Thr Glu Phe Gln Asn Val Gln
                325                 330                 335
Pro Thr Asn Glu Lys Met Thr Asp Leu Gln Asp Thr Lys Tyr Val Val
            340                 345                 350
Phe Glu Ser Val Glu Asn Asn Glu Ser Val Met Asp Thr Phe Val Lys
        355                 360                 365
His Pro Ile Lys Thr Gly Met Leu Asn Gly Lys Lys Tyr Val Val Met
    370                 375                 380
Glu Thr Thr Asn Asp Asp Tyr Trp Lys Asp Phe Ile Val Glu Gly Gln
385                 390                 395                 400
Arg Val Arg Thr Val Ser Lys Asp Ala Lys Asn Asn Thr Arg Thr Ile
                405                 410                 415
Ile Phe Pro Tyr Val Glu Gly Lys Thr Leu Tyr Asp Ala Ile Val Lys
            420                 425                 430
Val His Val Lys Thr Ile Asp Tyr Asp Gly Gln Tyr His Val Arg Ile
        435                 440                 445
```

```
Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr Asp Lys Ser Asn Lys
    450                 455                 460
Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu Ala Thr Pro Ala Thr
465                 470                 475                 480
Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys Glu Ser Gln Lys Gln
                485                 490                 495
Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro Ser Val Glu Lys Glu
            500                 505                 510
Asn Asp Ala Ser Ser Glu Ser Gly Lys Asp Lys Thr Pro Ala Thr Lys
        515                 520                 525
Pro Thr Lys Gly Glu Val Glu Ser Ser Ser Thr Thr Pro Thr Lys Val
    530                 535                 540
Val Ser Thr Thr Gln Asn Val Ala Lys Pro Thr Thr Ala Ser Ser Lys
545                 550                 555                 560
Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly Ser Ser Glu Ala Lys
                565                 570                 575
Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
            580                 585                 590
His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln Glu Asn Lys Ala Lys
        595                 600                 605
Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro
    610                 615                 620
Leu Met Ala Leu Leu Ala Leu Ser Ser Ile Val Ala Phe Val Leu Pro
625                 630                 635                 640
Arg Lys Arg Lys Asn
                645
```

<210> 16
<211> 645
<212> PRT
<213> Artificial Sequence

<220>
<223> ORF0657n mutant

<400> 16

```
Met Asn Lys Gln Gln Lys Glu Phe Lys Ser Phe Tyr Ser Ile Arg Lys
 1                5                  10                  15
Ser Ser Leu Gly Val Ala Ser Val Ala Ile Ser Thr Leu Leu Leu Leu
            20                  25                  30
Met Ser Asn Gly Glu Ala Gln Ala Ala Ala Glu Glu Thr Gly Gly Thr
        35                  40                  45
Asn Thr Glu Ala Gln Pro Lys Thr Glu Ala Val Ala Ser Pro Thr Thr
    50                  55                  60
Thr Ser Glu Lys Ala Pro Glu Thr Lys Pro Val Ala Asn Ala Val Ser
65                  70                  75                  80
Val Ser Asn Lys Glu Val Glu Ala Pro Thr Ser Glu Thr Lys Glu Ala
                85                  90                  95
Lys Glu Val Lys Glu Val Lys Ala Pro Lys Glu Thr Lys Glu Val Lys
            100                 105                 110
Pro Ala Ala Lys Ala Thr Asn Asn Thr Tyr Pro Ile Leu Asn Gln Glu
        115                 120                 125
Leu Arg Glu Ala Ile Lys Asn Pro Ala Ile Ile Asp Lys Asp His Ser
    130                 135                 140
```

```
Ala Pro Asn Ser Arg Pro Ile Asp Phe Glu Met Lys Lys Lys Asp Gly
145                 150                 155                 160
Thr Gln Gln Phe Tyr His Tyr Ala Ser Ser Val Lys Pro Ala Arg Val
                165                 170                 175
Ile Phe Thr Asp Ser Gly Pro Glu Ile Glu Leu Gly Leu Gln Ser Gly
            180                 185                 190
Gln Phe Trp Arg Lys Phe Glu Val Tyr Glu Gly Asp Lys Lys Leu Pro
        195                 200                 205
Ile Lys Leu Val Ser Tyr Asp Thr Val Lys Asp Tyr Ala Tyr Ile Arg
    210                 215                 220
Phe Pro Val Ser Asn Gly Thr Lys Ala Val Lys Ile Val Ser Ser Thr
225                 230                 235                 240
His Phe Asn Asn Lys Glu Glu Lys Tyr Asp Tyr Thr Leu Met Glu Phe
                245                 250                 255
Ala Gln Pro Ile Tyr Asn Ser Ala Asp Lys Phe Lys Thr Glu Glu Asp
            260                 265                 270
Tyr Lys Ala Glu Lys Leu Leu Ala Pro Tyr Lys Lys Ala Lys Thr Leu
        275                 280                 285
Glu Arg Gln Val Tyr Glu Leu Asn Lys Ile Gln Asp Lys Leu Pro Glu
    290                 295                 300
Lys Leu Lys Ala Glu Tyr Lys Lys Lys Leu Glu Asp Thr Lys Lys Ala
305                 310                 315                 320
Leu Asp Glu Gln Val Lys Ser Ala Ile Thr Glu Phe Gln Asn Val Gln
                325                 330                 335
Pro Thr Asn Glu Lys Met Thr Asp Leu Gln Asp Thr Lys Tyr Val Val
            340                 345                 350
Tyr Glu Ser Val Glu Asn Asn Glu Ser Met Met Asp Thr Phe Val Lys
        355                 360                 365
His Pro Ile Lys Thr Gly Met Leu Asn Gly Lys Lys Tyr Met Val Met
    370                 375                 380
Glu Thr Thr Asn Asp Asp Tyr Trp Lys Asp Phe Met Val Glu Gly Gln
385                 390                 395                 400
Arg Val Arg Thr Ile Ser Lys Asp Ala Lys Asn Asn Thr Arg Thr Ile
                405                 410                 415
Ile Phe Pro Tyr Val Glu Gly Lys Thr Leu Tyr Asp Ala Ile Val Lys
            420                 425                 430
Val His Val Lys Thr Ile Asp Tyr Asp Gly Gln Tyr His Val Arg Ile
        435                 440                 445
Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr Asp Lys Ser Asn Lys
    450                 455                 460
Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu Ala Thr Pro Ala Thr
465                 470                 475                 480
Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys Glu Ser Gln Lys Gln
                485                 490                 495
Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro Ser Val Glu Lys Glu
            500                 505                 510
Asn Asp Ala Ser Ser Glu Ser Gly Lys Asp Lys Thr Pro Ala Thr Lys
        515                 520                 525
Pro Thr Lys Gly Glu Val Glu Ser Ser Ser Thr Thr Pro Thr Lys Val
    530                 535                 540
Val Ser Thr Thr Gln Asn Val Ala Lys Pro Thr Thr Ala Ser Ser Lys
545                 550                 555                 560
Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly Ser Ser Glu Ala Lys
                565                 570                 575
```

```
Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
            580                 585                 590
His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln Glu Asn Lys Ala Lys
        595                 600                 605
Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro
    610                 615                 620
Leu Met Ala Leu Leu Ala Leu Ser Ser Ile Val Ala Phe Val Leu Pro
625                 630                 635                 640
Arg Lys Arg Lys Asn
                645
```

<210> 17
<211> 645
<212> PRT
<213> Artificial Sequence

<220>
<223> ORF0657n mutant

<400> 17

```
Met Asn Lys Gln Gln Lys Glu Phe Lys Ser Phe Tyr Ser Ile Arg Lys
 1               5                  10                  15
Ser Ser Leu Gly Val Ala Ser Val Ala Ile Ser Thr Leu Leu Leu Leu
            20                  25                  30
Met Ser Asn Gly Glu Ala Gln Ala Ala Ala Glu Glu Thr Gly Gly Thr
        35                  40                  45
Asn Thr Glu Ala Gln Pro Lys Thr Glu Ala Val Ala Ser Pro Thr Thr
    50                  55                  60
Thr Ser Glu Lys Ala Pro Glu Thr Lys Pro Val Ala Asn Ala Val Ser
65                  70                  75                  80
Val Ser Asn Lys Glu Val Glu Ala Pro Thr Ser Glu Thr Lys Glu Ala
                85                  90                  95
Lys Glu Val Lys Glu Val Lys Ala Pro Lys Glu Thr Lys Glu Val Lys
            100                 105                 110
Pro Ala Ala Lys Ala Thr Asn Asn Thr Tyr Pro Ile Leu Asn Gln Glu
        115                 120                 125
Leu Arg Glu Ala Ile Lys Asn Pro Ala Ile Ile Asp Lys Asp His Ser
    130                 135                 140
Ala Pro Asn Ser Arg Pro Ile Asp Phe Glu Met Lys Lys Lys Asp Gly
145                 150                 155                 160
Thr Gln Gln Phe Tyr His Tyr Ala Ser Ser Val Lys Pro Ala Arg Val
                165                 170                 175
Ile Phe Thr Asp Ser Gly Pro Glu Ile Glu Leu Gly Leu Gln Ser Gly
            180                 185                 190
Gln Phe Trp Arg Lys Phe Glu Val Tyr Glu Gly Asp Lys Lys Leu Pro
        195                 200                 205
Ile Lys Leu Val Ser Tyr Asp Thr Val Lys Asp Tyr Ala Tyr Ile Arg
    210                 215                 220
Phe Pro Val Ser Asn Gly Thr Lys Ala Val Lys Ile Val Ser Ser Thr
225                 230                 235                 240
His Phe Asn Asn Lys Glu Glu Lys Tyr Asp Tyr Thr Leu Met Glu Phe
                245                 250                 255
Ala Gln Pro Ile Tyr Asn Ser Ala Asp Lys Phe Lys Thr Glu Glu Asp
            260                 265                 270
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Tyr Lys Ala Glu Lys Leu Leu Ala Pro Tyr Lys Lys Ala Lys Thr Leu
275 280 285
Glu Arg Gln Val Tyr Glu Leu Asn Lys Ile Gln Asp Lys Leu Pro Glu
290 295 300
Lys Leu Lys Ala Glu Tyr Lys Lys Lys Leu Glu Asp Thr Lys Lys Ala
305 310 315 320
Leu Asp Glu Gln Val Lys Ser Ala Ile Thr Glu Phe Gln Asn Val Gln
325 330 335
Pro Thr Asn Glu Lys Met Thr Asp Leu Gln Asp Thr Lys Tyr Val Val
340 345 350
Tyr Glu Ser Glu Glu Asn Asn Glu Ser Met Met Asp Thr Phe Val Lys
355 360 365
His Pro Ile Tyr Thr Gly Met Leu Asn Gly Lys Lys Tyr Met Val Met
370 375 380
Glu Thr Thr Asn Asp Asp Tyr Trp Lys Asp Phe Met Val Glu Gly Gln
385 390 395 400
Arg Val Arg Thr Ile Ser Lys Asp Ala Lys Asn Asn Thr Arg Thr Ile
405 410 415
Ile Phe Pro Tyr Val Glu Gly Lys Thr Leu Tyr Asp Ala Ile Val Lys
420 425 430
Val His Val Lys Thr Ile Asp Tyr Asp Gly Gln Tyr His Val Arg Ile
435 440 445
Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr Asp Lys Ser Asn Lys
450 455 460
Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu Ala Thr Pro Ala Thr
465 470 475 480
Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys Glu Ser Gln Lys Gln
485 490 495
Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro Ser Val Glu Lys Glu
500 505 510
Asn Asp Ala Ser Ser Glu Ser Gly Lys Asp Lys Thr Pro Ala Thr Lys
515 520 525
Pro Thr Lys Gly Glu Val Glu Ser Ser Ser Thr Thr Pro Thr Lys Val
530 535 540
Val Ser Thr Thr Gln Asn Val Ala Lys Pro Thr Thr Ala Ser Ser Lys
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565 570 575
Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
580 585 590
His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln Glu Asn Lys Ala Lys
595 600 605
Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro
610 615 620
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625 630 635 640
Arg Lys Arg Lys Asn
645

<210> 18
<211> 645
<212> PRT
<213> Artificial Sequence

<220>

<223> ORF0657n mutant

<400> 18

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            20                  25                  30
Met Ser Asn Gly Glu Ala Gln Ala Ala Ala Glu Glu Thr Gly Gly Thr
        35                  40                  45
Asn Thr Glu Ala Gln Pro Lys Thr Glu Ala Val Ala Ser Pro Thr Thr
    50                  55                  60
Thr Ser Glu Lys Ala Pro Glu Thr Lys Pro Val Ala Asn Ala Val Ser
65                  70                  75                  80
Val Ser Asn Lys Glu Val Glu Ala Pro Thr Ser Glu Thr Lys Glu Ala
                85                  90                  95
Lys Glu Val Lys Glu Val Lys Ala Pro Lys Glu Thr Lys Glu Val Lys
            100                 105                 110
Pro Ala Ala Lys Ala Thr Asn Asn Thr Tyr Pro Ile Leu Asn Gln Glu
        115                 120                 125
Leu Arg Glu Ala Ile Lys Asn Pro Ala Ile Ile Asp Lys Asp His Ser
    130                 135                 140
Ala Pro Asn Ser Arg Pro Ile Asp Phe Glu Met Lys Lys Lys Asp Gly
145                 150                 155                 160
Thr Gln Gln Phe Tyr His Tyr Ala Ser Ser Val Lys Pro Ala Arg Val
                165                 170                 175
Ile Phe Thr Asp Ser Gly Pro Glu Ile Glu Leu Gly Leu Gln Ser Gly
            180                 185                 190
Gln Phe Trp Arg Lys Phe Glu Val Tyr Glu Gly Asp Lys Lys Leu Pro
        195                 200                 205
Ile Lys Leu Val Ser Tyr Asp Thr Val Lys Asp Tyr Ala Tyr Ile Arg
    210                 215                 220
Phe Pro Val Ser Asn Gly Thr Lys Ala Val Lys Ile Val Ser Ser Thr
225                 230                 235                 240
His Phe Asn Asn Lys Glu Glu Lys Tyr Asp Tyr Thr Leu Met Glu Phe
                245                 250                 255
Ala Gln Pro Ile Tyr Asn Ser Ala Asp Lys Phe Lys Asp Glu Glu Asp
            260                 265                 270
Tyr Lys Ala Glu Lys Leu Leu Ala Pro Tyr Lys Lys Ala Lys Thr Leu
        275                 280                 285
Glu Arg Gln Val Tyr Glu Leu Asn Lys Ile Gln Asp Lys Leu Pro Glu
    290                 295                 300
Lys Leu Lys Ala Glu Tyr Lys Lys Lys Leu Glu Asp Thr Lys Lys Ala
305                 310                 315                 320
Leu Asp Glu Gln Val Lys Ser Ala Ile Thr Glu Phe Gln Asn Val Gln
                325                 330                 335
Pro Thr Asn Glu Lys Met Thr Asp Leu Gln Asp Thr Lys Tyr Val Val
            340                 345                 350
Tyr Glu Ser Glu Glu Asn Asn Glu Ser Met Met Asp Thr Phe Val Lys
        355                 360                 365
His Pro Ile Tyr Thr Gly Met Leu Asn Gly Lys Lys Tyr Met Val Met
    370                 375                 380
Glu Thr Thr Asn Asp Asp Tyr Trp Lys Asp Phe Met Val Glu Gly Gln
385                 390                 395                 400
Arg Val Arg Thr Ile Ser Lys Asp Ala Lys Asn Asn Thr Arg Thr Ile
                405                 410                 415
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Ile Phe Pro Tyr Val Glu Gly Lys Thr Leu Tyr Asp Ala Ile Val Lys
                420                 425                 430
Val His Val Lys Thr Ile Asp Tyr Asp Gly Gln Tyr His Val Arg Ile
        435                 440                 445
Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr Asp Lys Ser Asn Lys
    450                 455                 460
Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu Ala Thr Pro Ala Thr
465                 470                 475                 480
Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys Glu Ser Gln Lys Gln
                485                 490                 495
Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro Ser Val Glu Lys Glu
            500                 505                 510
Asn Asp Ala Ser Ser Glu Ser Gly Lys Asp Lys Thr Pro Ala Thr Lys
        515                 520                 525
Pro Thr Lys Gly Glu Val Glu Ser Ser Ser Thr Thr Pro Thr Lys Val
    530                 535                 540
Val Ser Thr Thr Gln Asn Val Ala Lys Pro Thr Thr Ala Ser Ser Lys
545                 550                 555                 560
Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly Ser Ser Glu Ala Lys
                565                 570                 575
Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
            580                 585                 590
His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln Glu Asn Lys Ala Lys
        595                 600                 605
Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro
    610                 615                 620
Leu Met Ala Leu Leu Ala Leu Ser Ser Ile Val Ala Phe Val Leu Pro
625                 630                 635                 640
Arg Lys Arg Lys Asn
                645
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<210> 19
<211> 650
<212> PRT
<213> Artificial Sequence

<220>
<223> ORF0657n mutant

<400> 19

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Met Asn Lys Gln Gln Lys Glu Phe Lys Ser Phe Tyr Ser Ile Arg Lys
 1               5                  10                  15
Ser Ser Leu Gly Val Ala Ser Val Ala Ile Ser Thr Leu Leu Leu Leu
            20                  25                  30
Met Ser Asn Gly Glu Ala Gln Ala Ala Ala Glu Glu Thr Gly Gly Thr
        35                  40                  45
Asn Thr Glu Ala Gln Pro Lys Thr Glu Ala Val Ala Ser Pro Thr Thr
    50                  55                  60
Thr Ser Glu Lys Ala Pro Glu Thr Lys Pro Val Ala Asn Ala Val Ser
65                  70                  75                  80
Val Ser Asn Lys Glu Val Glu Ala Pro Thr Ser Glu Thr Lys Glu Ala
                85                  90                  95
Lys Glu Val Lys Glu Val Lys Ala Pro Lys Glu Thr Lys Glu Val Lys
            100                 105                 110
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Pro Ala Ala Lys Ala Thr Asn Asn Thr Tyr Pro Ile Leu Asn Gln Glu
        115                 120                 125
Leu Arg Glu Gly Ser Glu Ala Ile Lys Asn Pro Ala Ile Lys Asp Lys
    130                 135                 140
Asp His Ser Ala Pro Asn Ser Arg Pro Ile Asp Phe Glu Met Lys Lys
145                 150                 155                 160
Lys Asp Gly Thr Gln Gln Phe Tyr His Tyr Ala Ser Ser Val Lys Pro
                165                 170                 175
Ala Arg Val Ile Phe Thr Asp Ser Lys Pro Glu Ile Glu Leu Gly Leu
            180                 185                 190
Gln Ser Gly Gln Phe Trp Arg Lys Phe Glu Val Tyr Glu Gly Asp Lys
        195                 200                 205
Lys Leu Pro Ile Lys Leu Val Ser Tyr Asp Thr Val Lys Asp Tyr Ala
    210                 215                 220
Tyr Ile Arg Phe Ser Val Ser Asn Gly Thr Lys Ala Val Lys Ile Val
225                 230                 235                 240
Ser Ser Thr His Phe Asn Asn Lys Glu Glu Lys Tyr Asp Tyr Thr Leu
                245                 250                 255
Met Glu Phe Ala Gln Pro Ile Tyr Asn Ser Ala Asp Lys Phe Lys Thr
            260                 265                 270
Glu Glu Asp Tyr Lys Ala Glu Lys Leu Leu Ala Pro Tyr Lys Lys Ala
        275                 280                 285
Lys Thr Leu Glu Arg Gln Val Tyr Glu Leu Asn Lys Ile Gln Asp Lys
    290                 295                 300
Leu Pro Glu Lys Leu Lys Ala Glu Tyr Lys Lys Lys Leu Glu Asp Thr
305                 310                 315                 320
Lys Lys Ala Leu Asp Glu Gln Val Lys Ser Ala Ile Thr Glu Phe Gln
                325                 330                 335
Asn Val Gln Pro Thr Asn Glu Lys Met Thr Asp Leu Gln Asp Thr Lys
            340                 345                 350
Tyr Val Val Tyr Glu Ser Val Glu Asn Asn Glu Ser Met Met Asp Thr
        355                 360                 365
Phe Val Lys His Pro Ile Lys Thr Gly Met Leu Asn Gly Lys Lys Tyr
    370                 375                 380
Met Val Met Glu Thr Thr Asn Asp Asp Tyr Trp Lys Asp Phe Met Val
385                 390                 395                 400
Glu Gly Gln Arg Val Arg Thr Ile Ser Lys Asp Ala Lys Asn Asn Thr
                405                 410                 415
Arg Thr Ile Ile Phe Pro Tyr Val Glu Gly Lys Thr Leu Tyr Asp Ala
            420                 425                 430
Ile Val Lys Val His Val Lys Thr Ile Asp Tyr Asp Gly Gln Tyr His
        435                 440                 445
Val Arg Ile Val Asp Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr
    450                 455                 460
Asp Lys Ser Asn Lys Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu
465                 470                 475                 480
Ala Thr Pro Ala Thr Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys
                485                 490                 495
Glu Ser Gln Lys Gln Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro
            500                 505                 510
Ser Val Glu Lys Glu Asn Asp Ala Ser Ser Glu Ser Gly Lys Asp Lys
        515                 520                 525
Thr Pro Ala Thr Lys Pro Thr Lys Gly Glu Val Glu Ser Ser Ser Thr
    530                 535                 540
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Thr Pro Thr Lys Val Val Ser Thr Thr Gln Asn Val Ala Lys Pro Thr
545 550 555 560
Thr Ala Ser Ser Lys Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly
565 570 575
Ser Ser Glu Ala Lys Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys
580 585 590
Asn Thr Asn Asp Gly His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln
595 600 605
Glu Asn Lys Ala Lys Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys
610 615 620
Asp Met Thr Leu Pro Leu Met Ala Leu Leu Ala Leu Ser Ser Ile Val
625 630 635 640
Ala Phe Val Leu Pro Arg Lys Arg Lys Asn
645 650

<210> 20
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> 2H2 Vh

<400> 20
Asp Val His Leu Val Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
1 5 10 15
Asn Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Arg Tyr
20 25 30
Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45
Gly Leu Ile Trp Ala Gly Gly Val Thr Ile Tyr Asn Ser Thr Leu Met
50 55 60
Ser Arg Leu Ser Ile Ser Lys Asp Ser Ser Lys Ser Gln Val Phe Leu
65 70 75 80
Lys Met Asn Ser Leu Gln Ile Asp Asp Thr Ala Ile Tyr Tyr Cys Ala
85 90 95
Arg Glu Ala Ser Arg Asp His Tyr Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110
Thr Leu Thr Val Ser Ser
115

<210> 21
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> 2H2 Vl

<400> 21
Asp Ile Val Met Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15
Glu Lys Ile Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Ile
20 25 30

Tyr Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
35 40 45
Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Phe Arg Phe Ser Gly Gly
50 55 60
Gly Ser Gly Thr Ser Phe Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65 70 75 80
Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr
85 90 95
Phe Gly Ala Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 22
<211> 213
<212> PRT
<213> Artificial Sequence

<220>
<223> Mouse 2H2B3 Variable and Human Kappa Constant Region

<400> 22
Asp Ile Val Met Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15
Glu Lys Ile Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Ile
20 25 30
Tyr Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
35 40 45
Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Phe Arg Phe Ser Gly Gly
50 55 60
Gly Ser Gly Thr Ser Phe Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65 70 75 80
Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr
85 90 95
Phe Gly Ala Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210

<210> 23
<211> 448
<212> PRT
<213> Artificial Sequence

<220>
<223> Mouse 2H2B3 Variable and Human IgG1 Constant Region

<400> 23

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Asn	Leu	Ser	Ile	Thr	Cys	Thr	Val	Ser	Gly	Phe	Ser	Leu	Ser	Arg	Tyr
		20					25					30			
Gly	Val	His	Trp	Val	Arg	Gln	Pro	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Leu
	35					40					45				
Gly	Leu	Ile	Trp	Ala	Gly	Gly	Val	Thr	Ile	Tyr	Asn	Ser	Thr	Leu	Met
	50					55					60				
Ser	Arg	Leu	Ser	Ile	Ser	Lys	Asp	Ser	Ser	Lys	Ser	Gln	Val	Phe	Leu
65					70					75					80
Lys	Met	Asn	Ser	Leu	Gln	Ile	Asp	Asp	Thr	Ala	Ile	Tyr	Tyr	Cys	Ala
				85					90					95	
Arg	Glu	Ala	Ser	Arg	Asp	His	Tyr	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr
			100					105					110		
Thr	Leu	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro
		115					120					125			
Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly
	130					135					140				
Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn
145					150					155					160
Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln
				165					170					175	
Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser
			180					185					190		
Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser
		195					200					205			
Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr
	210					215					220				
His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser
225					230					235					240
Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg
				245					250					255	
Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro
			260					265					270		
Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala
		275					280					285			
Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val
	290					295					300				
Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr
305					310					315					320
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr
				325					330					335	
Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu
			340					345					350		
Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys
		355					360					365			
Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser
	370					375					380				
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp
385					390					395					400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
405 410 415
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
420 425 430
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 24
<211> 354
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence encoding 2H2 Vh

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acttgcactg tctctgggtt ctcattatcc cgctatggtg tacactgggt tcgccagcct 120
ccaggaaagg gtctggagtg gctgggacta atatgggctg gtggagtcac aatttataat 180
tcgactctca tgtccagact gagcatcagc aaagacagct ccaagagcca ggttttccta 240
aaaatgaaca gtctacaaat tgatgacaca gccatttact actgtgccag agaagcatct 300
cgggaccact actttgacta ctggggccaa ggcaccactc tcacagtctc ctcg 354

<210> 25
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence encoding 2H2 Vl

<400> 25
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atgacctgca gtgccagctc aagtgttagt tacatctact ggtaccagca gaagtcaggc 120
acctccccca aaagatggat ttatgacaca tccaaactgg cttctggagt ccctttttcgc 180
ttcagtggcg gtgggtctgg gacctctttc tctctcacaa tcagcagcat ggaggctgaa 240
gatgctgcca cttattactg ccagcagtgg agtagtaacc cactcacgtt cggtgctggg 300
accaagctgg aaataaaacg t 321

<210> 26
<211> 19
<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain leader sequence

<400> 26
Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
1 5 10 15
Val His Ser

<210> 27
<211> 20

<212> PRT
<213> Artificial Sequence

<220>
<223> Light chain leader sequence

<400> 27
Met Ser Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp Leu Thr
1 5 10 15
Asp Ala Arg Cys
20

<210> 28
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 28
acagatgcca gatgcgatat tgtgatgacc cagtct 36

<210> 29
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 29
tgcagccacc gtacgtttta tttccagctt ggtccc 36

<210> 30
<211> 36
<212> DNA
<213> Oligonucleotide Primer

<400> 30
acaggtgtcc actcggatgt gcacctggtg gagtca 36

<210> 31
<211> 36
<212> DNA
<213> Oligonucleotide Primer

<400> 31
gcccttggtg gatgccgagg agactgtgag agtggt 36

<210> 32
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 32
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15
Gly Gly Gly Ser
20

<210> 33
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 33
gtattaggaa ttcggccccc gaggccgagg 30

<210> 34
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 34
gcattactcg cggcccagcc ggccatggcg gac 33

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(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **BROWN, Martha, J.** [US/US]; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US). **ANDERSON, Annaliesa, S.** [GB/US]; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US). **COPE, Leslie, D.** [US/US]; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US). **JANSEN, Kathrin, Ute** [DE/US]; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US). **MCNEELY, Tessie** [US/US]; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US). **HARVEY, Barrett** [US/US]; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US). **DURR, Erberhard** [DE/US]; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US). **ERNST, Robin** [US/US]; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US).

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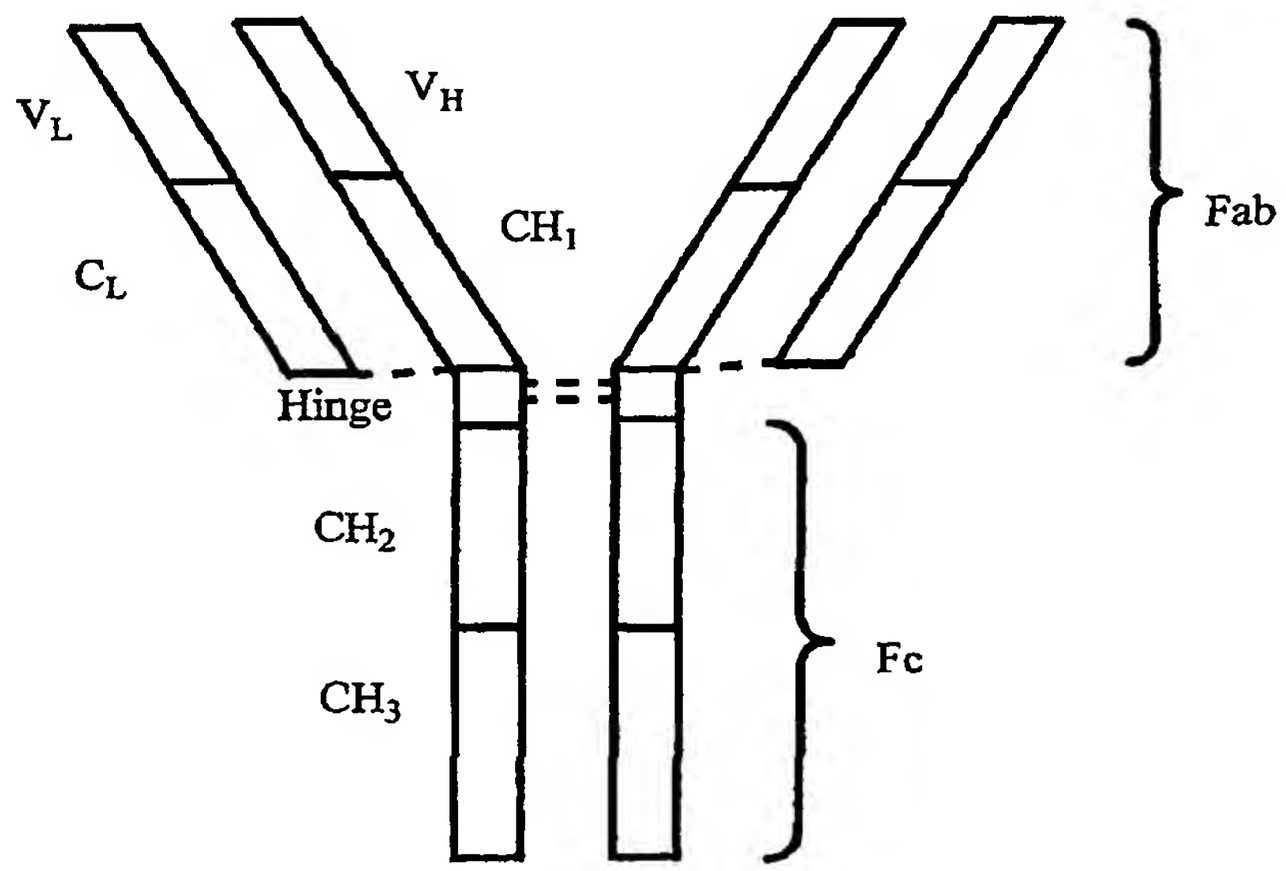
[Continued on next page]

(54) Title: ANTIGEN-BINDING PROTEINS TARGETING S. AUREUS ORF0657N



(57) Abstract: The present invention features antigen binding protein that bind an ORF0657n target region (SEQ ID NO: 1). ORF0657n is an *S. aureus* protein. ORF0657n target regions are provided by the mAb 1G3.BD4, mAb 2H2.BE11, mAb 13C7.BC1, and mAb 13G11.BF3 binding sites. In a lethal model challenge, mAb 2H2.BE11 and mAb 13C7.BC1 provided for increased survival against *S. aureus* infection. There was also protection demonstrated in an *ex vivo* model with either the IgG1 or the IgG2b form of mAb 2H2; and in a passive immunization murine indwelling catheter model using mAb 2H2.BE11.

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MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 07/01687

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/53; C12N 5/06, 5/16 (2007.01)
USPC - 435/7.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 435/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC: 435/7.2, 70.21, 188.5, 326-332, 345 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST (DB=PGPB,USPT,EPAB,JPAB) ,PubMed, Google, WIPO
Terms:ORF0657, antibody, Staphylococcus aureus, staphylococcal, monoclonal
mAb 1G3.BD4, mAb 2H2.BE11, mAb 13C7.BC1, mAb 13G11.BF3, hydridoma cell

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2005/009379 A2 (Anderson et al.) 03 February 2005 (03.02.2005), especially description, para [117]-[118]	1, 27-32 --- 2-13
X --- Y	US 6,979,446 B2 (Patti et al.) 27 December 2005 (27.12.2005), col 25, ln 44 to col 26, ln 1-6; col 4, ln 28-38; col 17, ln 65 to col 18, ln 34-44	14-18, 23, 25 --- 2-13, 19-22
Y	US 6,806,079 B1 (McCafferty et al.) October 19, 2004 (10.19.2004), pg 223,-224, SEQ ID NO 243	3-13, 19-22

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 August 2007 (06.08.2007)	21 NOV 2007
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 07/01687

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 24 and 26
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

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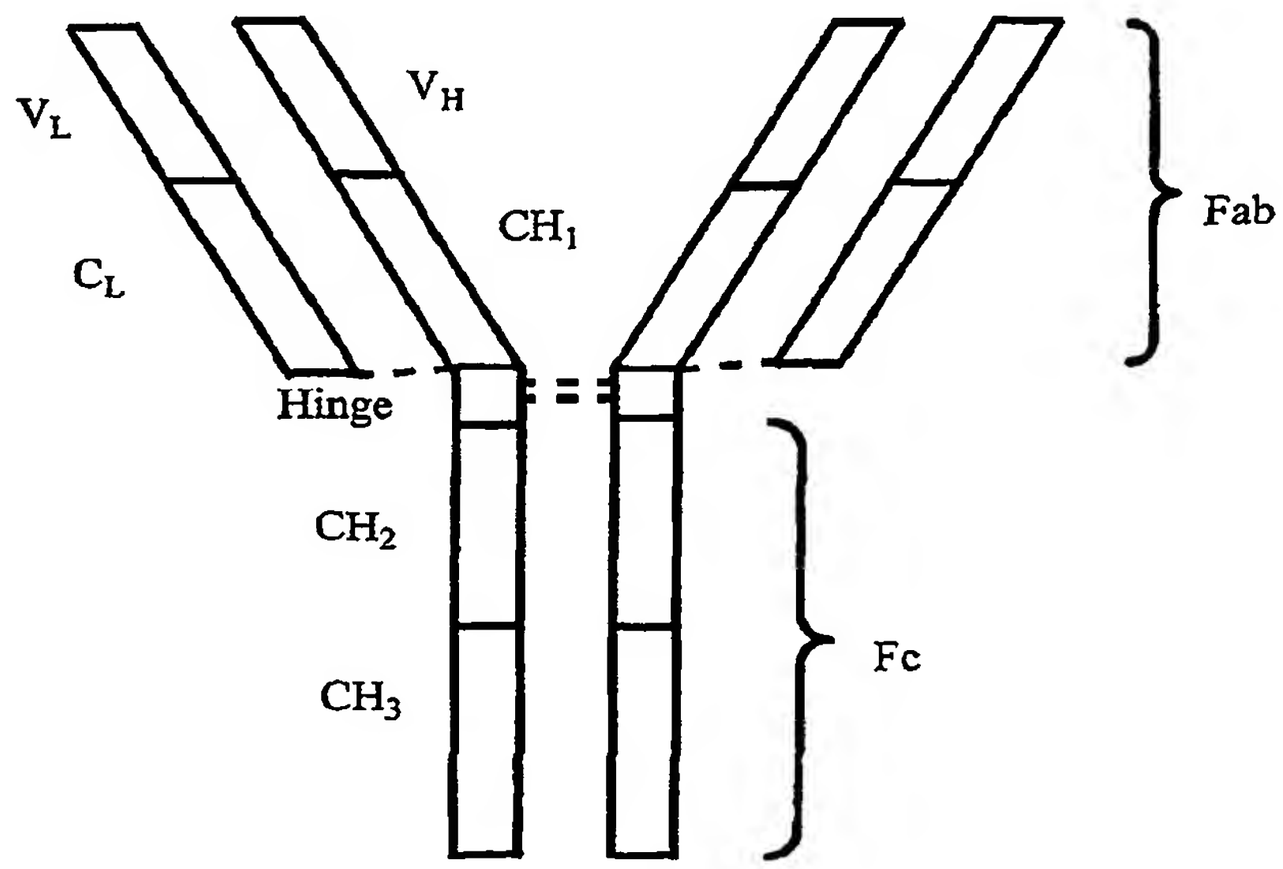
[Continued on next page]

(54) Title: ANTIGEN-BINDING PROTEINS TARGETING S. AUREUS ORF0657N



(57) Abstract: The present invention features antigen binding protein that bind an ORF0657n target region (SEQ ID NO: 1). ORF0657n is an *S. aureus* protein. ORF0657n target regions are provided by the mAb 1G3.BD4, mAb 2H2.BE11, mAb 13C7.BC1, and mAb 13G11.BF3 binding sites. In a lethal model challenge, mAb 2H2.BE11 and mAb 13C7.BC1 provided for increased survival against *S. aureus* infection. There was also protection demonstrated in an *ex vivo* model with either the IgG1 or the IgG2b form of mAb 2H2; and in a passive immunization murine indwelling catheter model using mAb 2H2.BE11.

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TITLE OF THE INVENTION

ANTIGEN-BINDING PROTEINS TARGETING S. AUREUS ORF0657n

RELATED APPLICATIONS

The present application claims priority to U.S. Provisional Application No. 60/763,023, filed January 27, 2006, which is hereby incorporated by reference herein.

BACKGROUND OF THE INVENTION

The references cited throughout the present application are not admitted to be prior art to the claimed invention.

Staphylococcus aureus is a pathogen responsible for a wide range of diseases and conditions. Examples of diseases and conditions caused by *S. aureus* include bacteremia, infective endocarditis, folliculitis, furuncle, carbuncle, impetigo, bullous impetigo, cellulitis, botryomyosis, toxic shock syndrome, scalded skin syndrome, central nervous system infections, infective and inflammatory eye disease, osteomyelitis and other infections of joints and bones, and respiratory tract infections. (*The Staphylococci in Human Disease*, Crossley and Archer (eds.), Churchill Livingstone Inc. 1997.)

Immunological based strategies can be employed to control *S. aureus* infections and the spread of *S. aureus*. Immunological based strategies include passive and active immunization. Passive immunization employs immunoglobulins targeting *S. aureus*. Active immunization induces immune responses against *S. aureus*.

SUMMARY OF THE INVENTION

The present invention features antigen binding protein that bind an ORF0657n target region (SEQ ID NO: 1). ORF0657n is an *S. aureus* protein. ORF0657n target regions are provided by the mAb 1G3.BD4, mAb 2H2.BE11, mAb 13C7.BC1, and mAb 13G11.BF3 binding sites. In a lethal model challenge, mAb 2H2.BE11 and mAb 13C7.BC1 provided for increased survival against *S. aureus* infection. There was also protection demonstrated in an *ex vivo* model with either the IgG1 or the IgG2b form of mAb 2H2; and in a passive immunization murine indwelling catheter model using mAb 2H2.BE11.

Mouse hybridoma cell lines producing mAb 1G3.BD4, mAb 2H2.BE11; mAb 13C7.BC1, and mAb 13G11.BF3 were deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, in accordance with Budapest Treaty on September 30, 2005. The cells lines were designated: ATCC No. PTA-7124 (producing mAb 2H2.BE11), ATCC No. PTA-7125 (producing mAb 13C7.BC1), ATCC No. PTA-7126 (producing mAb 1G3.BD4), and ATCC No. PTA-7127 (producing mAb 13G11.BF3).

Thus, a first aspect of the present invention features an isolated antigen binding protein comprising a first variable region and a second variable region. The first and second variable regions

bind one or more target regions selected from the group consisting of: mAb 1G3.BD4 target region, mAb 2H2.BE11 target region, mAb 13C7.BC1 target region, and mAb 13G11.BF3 target region.

Reference to "isolated" indicates a different form than found in nature. The different form can be, for example, a different purity than found in nature and/or a structure that is not found in nature. A structure not found in nature includes recombinant structures where different regions are combined together, for example, humanized antibodies where one or more murine complementary determining regions is inserted onto a human framework scaffold or a murine antibody is resurfaced to resemble the surface residues of a human antibody, hybrid antibodies where one or more complementary determining regions from an antigen binding protein is inserted into a different framework scaffold, and antibodies derived from natural human sequences where genes coding for light and heavy variable domains were randomly combined together.

The isolated protein is preferably substantially free of serum proteins. A protein substantially free of serum proteins is present in an environment lacking most or all serum proteins.

A "variable region" has the structure of an antibody variable region from a heavy or light chain. Antibody heavy and light chain variable regions contain three complementary determining regions interspaced onto a framework. The complementary determining regions are primarily responsible for recognizing a particular epitope.

A target region is defined with respect to the ORF0657n region (SEQ ID NO: 1) bound by mAb 1G3.BD4, mAb 2H2.BE11, mAb 13C7.BC1, or mAb 13G11.BF3. For example, the mAb IG3.BD4 target region is the ORF0657n region to which mAb IG3.BD4 binds.

A protein binding an identified target region competes with either mAb 1G3.BD4, mAb 2H2.BE11, mAb 13C7.BC1, or mAb 13G11.BF3 for binding to the target region. For example, a protein competing with mAb 1G3.BD4 binding to ORF0657n binds to the mAb 1G3.BD4 target region.

A protein that competes with either the monoclonal antibody mAb 1G3.B3, mAb 2H2.B8, mAb 13C7.D12, or mAb 13G11.C11 reduces binding of the monoclonal antibody to ORF0657n by at least about 20%, preferably at least about 50%, when excess and equal amounts of the competing protein and monoclonal antibody are employed.

Reference to "protein" indicates a contiguous amino acid sequence and does not provide a minimum or maximum size limitation. One or more amino acids present in the protein may contain a post-translational modification, such as glycosylation or disulfide bond formation.

A preferred antigen binding protein is a monoclonal antibody. Reference to a "monoclonal antibody" indicates a collection of antibodies having the same, or substantially the same, complementary determining region, and binding specificity. The variation in the antibodies is that which would occur if the antibodies were produced from the same construct(s).

Monoclonal antibodies can be produced, for example, from a particular hybridoma and from a recombinant cell containing one or more recombinant genes encoding the antibody. The antibody

may be encoded by more than one recombinant gene where, for example, one gene encodes the heavy chain and one gene encodes the light chain.

Another aspect of the present invention describes a nucleic acid containing a recombinant gene comprising a nucleotide sequence encoding an antibody variable region. The antibody variable region can bind a target region selected from the group consisting of: mAb IG3.BD4 target region, mAb 2H2.BE11 target region, mAb 13C7.BC1, and mAb 13G11.BF3 target region.

A recombinant gene contains recombinant nucleic acid encoding a protein along with regulatory elements for proper transcription and processing (which may include translational and post translational elements). The recombinant nucleic acid by virtue of its sequence and/or form does not occur in nature. Examples of recombinant nucleic acid include purified nucleic acid, two or more nucleic acid regions combined together providing a different nucleic acid than found in nature, and the absence of one or more nucleic acid regions (*e.g.*, upstream or downstream regions) that are naturally associated with each other.

Another aspect of the present invention describes a recombinant cell comprising one or more recombinant genes encoding an antibody variable region that binds to a target region selected from the group consisting of: mAb IG3.BD4 target region, mAb 2H2.BE11 target region, mAb 13C7.BC1, and mAb 13G11.BF3 target region. Multiple recombinant genes are useful, for example, where one gene encodes an antibody heavy chain or fragment thereof containing the V_h region and another nucleic acid encodes an antibody light chain or fragment thereof containing the V_l region.

Another aspect of the present invention comprises a method of producing a protein comprising an antibody variable region. The method comprising the steps of: (a) growing a recombinant cell comprising recombinant nucleotide acid encoding for a protein under conditions wherein the protein is expressed; and (b) purifying the protein.

Another aspect of the present invention describes a pharmaceutical composition. The composition contains a therapeutically effective amount of an antigen binding protein and a pharmaceutically acceptable carrier.

A therapeutically effective amount is an amount sufficient to provide a useful therapeutic or prophylactic effect. For a patient infected with *S. aureus*, an effective amount is sufficient to achieve one or more of the following effects: reduce the ability of *S. aureus* to propagate in the patient or reduce the amount of *S. aureus* in the patient. For a patient not infected with *S. aureus*, an effective amount is sufficient to achieve one or more of the following: a reduced susceptibility to *S. aureus* infection or a reduced ability of the infecting bacterium to establish persistent infection for chronic disease.

Another aspect of the present invention describes a method of detecting the presence of an OFR0657n antigen in a solution or on a cell. The method involves providing a binding protein described herein to the solution or cell and measuring the ability of the binding protein to bind to the antigen in the solution or cell. Measurements can be quantitative or qualitative.

Reference to ORF0657n antigen includes full-length ORF0657n or a derivative thereof having an epitope that is recognized by mAb 1G3.B3, mAb 2H2.B8, mAb 13C7.D12, or mAb 13G11.C11. Examples of derivatives include truncated versions; and full-length or truncated versions of ORF0657n containing one or more of the following amino acid alterations: one or more additions, one or more substitutions, and one or more deletions.

Another aspect of the present invention features a method of treating a patient against a *S. aureus* infection. The method comprises the step of administering to the patient an effective amount of an antigen binding protein described herein. The patient being treated may, or may not, be infected with *S. aureus*. Preferably, the patient is a human.

Another aspect of the present invention describes a cell line producing a protein that is either mAb 1G3.B3, mAb 2H2.B8, mAb 13C7.D12, or mAb 13G11.C11, or that competes with either mAb IG3.B3, mAb 2H2.B8, mAb 13C7.D12, or mAb 13G11.C11 for binding to ORF0657n. Preferred cells lines are hybridomas, and recombinant cell lines containing recombinant nucleic acid encoding the protein.

Reference to open-ended terms such as "comprises" allows for additional elements or steps. Occasionally phrases such as "one or more" are used with or without open-ended terms to highlight the possibility of additional elements or steps.

Unless explicitly stated reference to terms such as "a" or "an" is not limited to one. For example, "a cell" does not exclude "cells". Occasionally phrases such as one or more are used to highlight the possible presence of a plurality.

Other features and advantages of the present invention are apparent from the additional descriptions provided herein including the different examples. The provided examples illustrate different components and methodology useful in practicing the present invention. The examples do not limit the claimed invention. Based on the present disclosure the skilled artisan can identify and employ other components and methodology useful for practicing the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the structure of an IgG molecule. "V_L" refers to a light chain variable region. "V_H" refers to a heavy chain variable region. "C_L" refers to a light chain constant region. "CH_1", "CH_2" and "CH_3" are heavy chain constant regions. Dashed lines indicate disulfide bonds.

Figure 2 illustrates a matrix outlining the reactivities of different monoclonal antibodies in a pair-wise binding study. The panel of monoclonal antibodies fell into three reactive areas by the BIACORE® method.

Figures 3A-3C: Groups of BALB/c mice (n = 20) were treated 20 hours prior to bacterial challenge with an i.p. injection of: ■, mAb 13C7.BC1; □, mAb 6G6.A8 (isotype control); or ○, PBS. Mice were challenged with *S. aureus* by i.v. injection and survival was monitored. Fig. 3A-

0.49 mg mAb 13C7.BC1; 0.45 mg mAb 6G6.A8; and 9.8 x 10^8 CFU *S. aureus* Becker. Fig. 3B- 0.49 mg mAb 13C7.BC1; 0.45 mg mAb 6G6.A8; and 9.6 x 10^8 CFU *S. aureus* Becker. Fig. 3C- 0.50 mg mAb13C7.BC1; 0.45 mg mAb 6G6; and 9.9 x 10^8 CFU *S. aureus* Becker.

Figures 4A and 4B: Groups of BALB/c mice (n = 20) were treated 20 hours prior to bacterial challenge with an i.p. injection of: ■, mAb 13C7.BC1 (0.5 mg); □, mAb 6G6.A8 (isotype control) (0.5 mg); or ○, PBS (0.5 ml). Mice were challenged with *S. aureus* by i.v. injection and survival was monitored. Fig. 4A illustrates results with 2.09 X 10^8 CFU *S. aureus* UK58. Fig. 4B illustrates results with 2.15 X 10^8 *S. aureus* UK 58.

Figures 5A-5C: Groups of BALB/c mice (n = 20) were treated 20 hours prior to bacterial challenge with an i.p. injection of: ■, mAb 2H2.BE11, □, mAb 6G6.A8 (isotype control); ○, PBS. Mice were challenged with *S. aureus* by i.v. injection and survival was monitored. Fig. 5A- 0.43 mg mAb 2H2.BE11; 0.5 mg mAb 6G6.A8; and 9.8 x 10^8 CFU *S. aureus* Becker. Fig. 5B- 0.43 mg mAb 2H2.BE11; 0.5 mg mAb 6G6.A8; and 8.3 x 10^8 CFU *S. aureus* Becker. Fig. 5C- 0.43 mg mAb 2H2.BE11; 0.5 mg mAb 6G6.A8; and 9.3 x 10^8 CFU *S. aureus* Becker.

DETAILED DESCRIPTION OF THE INVENTION

ORF0657n is an *S. aureus* protein located at the *S. aureus* outer membrane. ORF0657n has been found to be well conserved in different strains of *S. aureus*. (Anderson *et al.*, International Publication No. WO 2005/009379, International Publication Date February 3, 2005.) Different ORF0657n derivatives can be used to produce a protective immune response against *S. aureus* infection. (Anderson *et al.*, International Publication No. WO 2005/009379, International Publication Date February 3, 2005.)

Due to their ability to recognize ORF0657n, the antigen binding proteins described herein can be used, for example, as a tool in the production, characterization, or study of ORF0657n based antigens. Antigen binding protein recognizing appropriate ORF0657n epitopes can also be used agent to treat *S. aureus* infection.

I. Antigen Binding Protein

Antigen binding proteins contain an antibody variable region providing for specific binding to an epitope. The antibody variable region can be present in, for example, a complete antibody, an antibody fragment, and a recombinant derivative of an antibody or antibody fragment.

Different classes of antibodies have different structures. Different antibody regions can be illustrated by reference to IgG (Figure 1). An IgG molecule contains four amino acid chains: two longer length heavy chains and two shorter light chains. The heavy and light chains each contain a constant region and a variable region. Within the variable regions are three hypervariable regions responsible for antigen specificity. (See, for example, Breitling *et al.*, Recombinant Antibodies, John Wiley & Sons, Inc. and Spektrum Akademischer Verlag, 1999; and Lewin, Genes IV, Oxford University Press and Cell Press; 1990.)

The hypervariable regions (also referred to as complementarity determining regions), are interposed between more conserved flanking regions (also referred to as framework regions). Amino acids associated with framework regions and complementarity determining regions can be numbered and aligned as described by Kabat *et al.*, Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services, 1991.

The two heavy chain carboxyl regions are constant regions joined by disulfide binding to produce an Fc region. The Fc region is important for providing antibody biological activity such as complement and macrophage activation. Each of the two heavy chains making up the Fc region extend into different Fab regions through a hinge region.

In higher vertebrates there are two classes of light chains and five classes of heavy chains. The light chains are either κ or λ. The heavy chains define the antibody class and are either α, δ, ε, γ, or μ. For example, IgG has a γ heavy chain. Subclasses also exist for different types of heavy chains such as human γ_1, γ_2, γ_3, and γ_4. Heavy chains impart a distinctive conformation to hinge and tail regions. (Lewin, Genes IV, Oxford University Press and Cell Press, 1990.)

Antibody fragments containing an antibody variable region include Fv, Fab, and Fab_2 regions. Each Fab region contains a light chain made up of a variable region and a constant region, and a heavy chain region containing a variable region and a constant region. A light chain is joined to a heavy chain by disulfide bonding through constant regions. The light and heavy chain variable regions of a Fab region provide for an Fv region that participates in antigen binding.

The antibody variable region can be present in a recombinant derivative. Examples of recombinant derivatives include single-chain antibodies, diabody, triabody, tetrabody, and miniantibody. (Kipriyanov *et al, Molecular Biotechnology 26*:39-60, 2004.)

The antigen binding protein can contain one or more variable regions recognizing the same or different epitopes. (Kipriyanov *et al., Molecular Biotechnology 26*:39-60, 2004.)

II. Generation of Antigen Binding Protein Directed to an Identified Target Region

Different antigen binding proteins directed to the mAb 1G3.BD4 target region, mAb 2H2.BE11 target region, mAb 13C7.BC1 target region, or mAb 13G11.BF3 target region can be generated starting with the respective monoclonal antibody. Alternatively, the epitope recognized by a binding protein can be used to select additional binding proteins.

The mAb 2H2.BE11 target region appears to be located at approximately amino acids 76-357 of ORF0657n. A polypeptide containing amino acids 76-357 of ORF0657n, or a full-length ORF0657n, can be used as a target antigen to select for antibodies. The target region of the generated antibodies can be determined.

A variety of techniques are available to select for a protein recognizing an antigen. Examples of such techniques include use of phage display technology and hybridoma production. Human antibodies can be produced using chimeric mice such as a XenoMouse or Trans-Chromo mouse.

(*E.g.*, Azzazy *et al.*, *Clinical Biochemistry 35*:425-445, 2002, Berger *et al.*, *Am. J. Med. Sci. 324(1)*:14-40, 2002.)

The monoclonal antibodies mAb 1G3.BD4, mAb 2H2.BE11, mAb 13C7.BC1, and mAb 13G11.BF3 contain variable regions recognizing ORF0675n. Additional binding proteins recognizing ORF0657n can be produced based on antibody variable regions. Additional binding proteins can, for example, be produced by modifying an existing monoclonal antibody and by using variable region sequence information. Protein construction and sequence manipulation can be performed using recombinant nucleic acid techniques.

The monoclonal antibodies mAb 1G3.BD4, mAb 2H2.BE11, mAb 13C7.BC1, and mAb 13G11.BF3 are murine antibodies. For human therapeutic applications, preferred binding proteins based on such mAb's are designed to reduce the potential generation of human anti-mouse antibodies recognizing the murine regions.

The potential generation of human anti-mouse antibodies can be reduced using techniques such as murine antibody humanization, de-immunization, and chimeric antibody production. (See, for example, O'Brien *et al.*, Humanization of Monoclonal Antibodies by CDR Grafting, p 81-100, From *Methods* in Molecular Biology Vol. 207: Recombinant antibodies for Cancer Therapy: Methods and Protocols (Eds. Welschof and Krauss) Humana Press, Totowa, New Jersey, 2003; Kipriyanov *et al.*, *Molecular Biotechnology 26*:39-60, 2004; Gonzales *et al.*, *Tumor Biol. 26*:31-43, 2005, Presta, *Advanced Drug Delivery Reviews 58*:640-656, 2006, Tsurushita *et al.*, *Methods 36*:69-83, 2005, Roque *et al.*, *Biotechnol. Prog. 20*:639-654, 2004.)

Murine antibodies can be humanized using techniques such as grafting complementary determining regions into a framework region or resurfacing. Resurfacing (also known as veneering) involves modifying a variable region so the surface exposed regions are humanized.

Grafting complementary determining regions involves taking such regions or a portion of such regions from, for example, a murine source and inserting the regions into a human variable region framework. The human framework used for grafting can be selected based on sequence homology to the variable region *(e.g.*, murine) from which the region was obtained. Essential framework residues associated with grafted complementary determining regions should also be provided in the new framework.

De-immunization involves altering potential linear T-cell epitopes present in the antibody. The epitopes can be identified based on a bioinformatics scan of know human HLA class I and/or class II epitopes. (Presta, *Advanced Drug Delivery Reviews 58*:640-656, 2006.)

A chimeric antibody contains a human constant region along with a variable region from a different organism, such as a mouse. The human constant region provides an Fc region.

Additional examples of alterations include providing a variable region in, for example, a single chain antibody, a diabody, a triabody, a tetrabody, and a miniantibody. (Kipriyanov *et al.*, *Molecular Biotechnology 26*:39-60, 2004.) The antigen binding protein can contain one or more variable

regions recognizing the same or different epitopes. (*Id.*) Additional embodiments of the present invention are directed to a single chain antibody, a diabody, a triabody, a tetrabody, or a miniantibody directed to the mAb 1G3.BD4, mAb 2H2.BE11, mAb 13C7.BC1, or mAb 13G11.BF3 binding site.

III. Binding Protein Directed to the mAb 2H2.BE11 Target Region

As described in the Examples provided below, the mAb 2H2.BE11 target region was further characterized and the amino acids sequence of the variable regions was determined. The identified target region and the sequence information facilitate obtaining different binding proteins directed to the mAb 2H2.BE11 target region.

In an embodiment of the present invention, the binding protein binds to a polypeptide consisting of amino acids 76-357 of SEQ ID NO: 1. Preferably, the binding protein is either a human antibody, a humanized antibody, a de-immunized antibody, or chimeric antibody. Preferred antibodies are isolated antibodies and monoclonal antibodies.

The amino acids sequences of the mAb 2H2.BE11 variable regions are provided by SEQ ID NO: 20 (V_h) and SEQ ID NO: 21 (V_l). The complementary determining regions (CDR's) within V_h were identified at amino acids 36-45, 50-65, and 98-107. The CDR's within V_l were identified at amino acids 24-33, 49-55, and 88-96 of SEQ ID NO: 21.

In different embodiments directed to a V_h region, the binding protein binds the mAb 2H2.BE11 target region and comprises, consists, or consists essentially of: a first V_h CDR comprising, consisting, or consisting essentially of amino acids 36-45 of SEQ ID NO: 20 or a sequence differing from amino acids 36-45 by one amino acid; a second V_h CDR comprising, consisting, or consisting essentially of amino acids 50-65 of SEQ ID NO: 20 or a sequence differing from amino acids 50-65 by one amino acid; and a third V_h CDR comprising, consisting, or consisting essentially of amino acids 98-107 of SEQ ID NO: 20 or a sequence differing from amino acids 98-107 by one amino acid.

In different embodiments directed to a V_l region, the binding protein binds the mAb 2H2.BE11 target region and comprises, consists, or consists essentially of a first V_l CDR comprising, consisting, or consisting essentially of amino acids 24-33 of SEQ ID NO: 21 or a sequence differing from amino acids 24-33 by one amino acid; a second V_l CDR comprising, consisting, or consisting essentially of amino acids 49-55 of SEQ ID NO: 21 or a sequence differing from amino acids 49-55 by one amino acid; and a third V_l CDR comprising, consisting, or consisting essentially of amino acids 88-96 of SEQ ID NO: 21 or a sequence differing from amino acids 88-96 by one amino acid.

Reference to "consisting essentially of" with respect to a variable region, CDR region, or antibody sequence, indicates the possible presence of one or more additional amino acids, where such amino acids do not significantly decrease binding to the target.

An amino acid difference can be an amino acid deletion, insertion, or substitution. In substituting amino acids to maintain activity, the substituted amino acids should have one or more similar properties such as approximately the same charge, size, polarity and/or hydrophobicity.

Preferably, an amino acid substitution is a conservative substitution. A conservative substitution replaces an amino acid with another amino acid having similar properties. Table 1 provides a list of groups of amino acids, where one member of the group is a conservative substitution for another member.

Table 1 : Conservative Substitutions

Ala, Val, Ile, Leu, Met
Ser, Thr,
Tyr, Trp
Asn, Gln
Asp, Glu
Lys, Arg, His

In additional embodiments the V_h region is either SEQ ID NO: 20, a humanized SEQ ID NO: 20 , or a de-immunized SEQ ID NO: 20; and/or the V_l region is either SEQ ID NO: 21, a humanized SEQ ID NO: 21, or a de-immunized SEQ ID NO: 21.

In different embodiments focusing on an antibody, the antibody comprises, consists, or consists essentially of: (a) a heavy chain comprising a V_h region as described in this Section III, and a human hinge, CH_1, CH_2, and CH_3 regions from an IgG_1, IgG_2, IgG_3 or IgG_4, and (b) a light chain comprising a V_l region as described above in this section III, and a human kappa C_L or human lambda C_L. In further embodiments: the antibody comprises, consists, or consists essentially of: (a) a heavy chain comprising a V_h region as described in this Section III, and a human hinge, CH_1, CH_2, and CH_3 regions from an IgG_1 or IgG_2 and (b) a light chain comprising a V_l region as described above in this Section III, and a human kappa C_L; and the heavy chain consists essentially of the amino acid sequence of SEQ ID NO: 22 and/or the light chain consists essentially of the amino acid sequence of SEQ ID NO: 23.

In additional embodiments the antigen-binding protein described herein has V_h and V_l regions providing an affinity K_D at least about 100 nM, preferably at least about 30 nM to the target antigen. Binding to the target antigen can be determined as described in Example 11, using an ORF0657n fragment from amino acids 42-486

Preferred binding proteins for the different embodiments are an antibody. More preferably the antibody is isolated or a monoclonal antibody.

IV. Protein Production

Antigen binding protein are preferably produced using recombinant nucleic acid techniques or through the use of a hybridoma. Recombinant nucleic acid techniques involve constructing

a nucleic acid template for protein synthesis. Hybridoma techniques involve using an immortalized cell line to produce the antigen binding protein. Suitable recombinant nucleic acid and hybridoma techniques are well known in the art. (See for example, Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 2005, Harlow *et al.*, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988.)

Recombinant nucleic acid encoding an antigen binding protein can be expressed in a host cell that in effect serves as a factory for the encoded protein. The recombinant nucleic acid can provide a recombinant gene encoding the antigen binding protein that exists autonomously from a host cell genome or as part of the host cell genome.

A recombinant gene contains nucleic acid encoding a protein along with regulatory elements for protein expression. Generally, the regulatory elements that are present in a recombinant gene include a transcriptional promoter, a ribosome binding site, a terminator, and an optionally present operator. A preferred element for processing in eukaryotic cells is a polyadenylation signal. Antibody associated introns may also be present. Examples of expression cassettes for antibody or antibody fragment production are well known in art. (*E.g.*, Persic *et al.*, *Gene 187*:9-18, 1997, Boel *et al.*, *J. Immunol. Methods 239*:153-166, 2000, Liang *et al.*, *J. Immunol. Methods* 247:119-130, 2001, Tsurushita *et al.*, *Methods 36*:69-83, 2005.)

Due to the degeneracy of the genetic code, a large number of different encoding nucleic acid sequences can be used to code for a particular protein. The degeneracy of the genetic code arises because almost all amino acids are encoded by different combinations of nucleotide triplets or "codons". Amino acids are encoded by codons as follows:

A=Ala=Alanine: codons GCA, GCC, GCG, GCU
C=Cys=Cysteine: codons UGC, UGU
D=Asp=Aspartic acid: codons GAC, GAU
E=Glu=Glutamic acid: codons GAA, GAG
F=Phe=Phenylalanine: codons UUC, UUU
G=Gly=Glycine: codons GGA, GGC, GGG, GGU
H=His=Histidine: codons CAC, CAU
I=Ile=Isoleucine: codons AUA, AUC, AUU
K=Lys=Lysine: codons AAA, AAG
L=Leu=Leucine: codons UUA, UUG, CUA, CUC, CUG, CUU
M=Met=Methionine: codon AUG
N=Asn=Asparagine: codons AAC, AAU
P=Pro=Proline: codons CCA, CCC, CCG, CCU
Q=Gln=Glutamine: codons CAA, CAG
R=Arg=Arginine: codons AGA, AGG, CGA, CGC, CGG, CGU
S=Ser=Serine: codons AGC, AGU, UCA, UCC, UCG, UCU
T=Thr=Threonine: codons ACA, ACC, ACG, ACU

V=Val=Valine: codons GUA, GUC, GUG, GUU

W=Trp=Tryptophan: codon UGG

Y=Tyr=Tyrosine: codons UAC, UAU

Expression of a recombinant gene in a cell is facilitated using an expression vector. Preferably, the expression vector, in addition to a recombinant gene, also contains an origin of replication for autonomous replication in a host cell, a selectable marker, a limited number of useful restriction enzyme sites, and a potential for high copy number. Examples of expression vectors for antibody and antibody fragment production are well known in art. (*E.g.*, Persic *et al., Gene 187*:9-18, 1997, Boel *et al., J. Immunol. Methods 239*:153-166, 2000, Liang *et al., J. Immunol. Methods* 247:119-130, 2001, Tsurushita *et al., Methods 36*:69-83, 2005.)

If desired, nucleic acid encoding an antibody may be integrated into the host chromosome using techniques well known in the art. (*E.g.*, Ausubel, *Current Protocols in Molecular Biology,* John Wiley, 2005, Marks *et al.*, International Application Number WO 95/17516, International Publication Date June 29, 1995.)

A variety of different cell lines can be used for recombinant antigen binding protein expression, including those from prokaryotic organisms (*e.g., E. coli, Bacillus sp,* and *Streptomyces sp.* (or streptomycete) and from eukaryotic (*e.g.*, yeast, Baculovirus, and mammalian). (Breitling *et al.*, Recombinant Antibodies, John Wiley & Sons, Inc. and Spektrum Akademischer Verlag, 1999, Kipriyanov *et al., Molecular Biotechnology 26*:39-60, 2004, Tsurushita *et al., Methods 36*:69-83, 2005.)

Preferred hosts for recombinant antigen binding protein expression provide for mammalian post translational modifications. Post translational modifications chemical modification such as glycosylation and disulfide bond formation. Another type of post translational modification is signal peptide cleavage.

Proper glycosylation can be important for antibody function. (Yoo *et al., Journal of Immunological Methods 261*:1-20, 2002, Li *et al., Nature Biotechnology 24*(2):210-215, 2006.) Naturally occurring antibodies contain at least one N-linked carbohydrate attached to a heavy chain. (Yoo *et al., Journal of Immunological Methods 261*:1-20, 2002.) Additional N-linked carbohydrates and O-linked carbohydrates may be present and may be important for antibody function. (*Id.*)

Different types of host cells can be used to provide for efficient post-translational modifications including mammalian host cells and non-mammalian cells. Examples of mammalian host cells include but are not limited to Chinese hamster ovary (Cho), HeLa, C6, PC12, Human Embryonic Kidney (HEK293) and myeloma cells. (Yoo *et al., Journal of Immunological Methods 261*:1-20, 2002, Persic *et al., Gene 187*:9-18, 1997.) Non-mammalian cells can be modified to replicate human glycosylation. (Li *et al., Nature Biotechnology 24*(2):210-215, 2006.) Glycoenginnered *Pichia pastoris* is an example of such a modified non-mammalian cell. (Li *et al., Nature Biotechnology 24*(2):210-215, 2006.)

Preferred recombinant genes comprise a nucleotide sequence encoding an antibody variable region that binds to a target region selected from the group consisting of: mAb IG3.BD4 target region, mAb 2H2.BE11 target region, mAb 13C7.BC1, and mAb 13G11.BF3 target region. A particular recombinant gene can encode for a protein containing one variable region or both a V_h and V_l region. The recombinant gene can also encode for antibody constant regions and hinge region. If desired, an antibody can be produced using a combination of recombinant genes, where one gene encodes for a light chain and the second gene encodes for a heavy chain.

Different embodiments are provided by the nucleic acid encoding a protein described in Section II or III *supra*. Examples of such embodiments are provided below.

In an embodiment directed to a V_h encoding region, the nucleotide sequence encodes a variable region comprising, consisting, or consisting essentially of: a first V_h CDR comprising, consisting, or consisting essentially of amino acids 36-45 of SEQ ID NO: 20 or a sequence differing from amino acids 36-45 by one amino acid; a second V_h CDR comprising, consisting, or consisting essentially of amino acids 50-65 of SEQ ID NO: 20 or a sequence differing from amino acids 50-65 by one amino acid; and a third V_h CDR comprising, consisting, or consisting essentially of amino acids 98-107 of SEQ ID NO: 20 or a sequence differing from amino acids 98-107 by one amino acid.

In an embodiment directed to a V_l encoding region, the nucleotide sequence encodes a variable region comprising, consisting, or consisting essentially of a first V_l CDR comprising, consisting, or consisting essentially of amino acids 24-33 of SEQ ID NO: 21 or a sequence differing from amino acids 24-33 by one amino acid; a second V_l CDR comprising, consisting, or consisting essentially of amino acids 49-55 of SEQ ID NO: 21 or a sequence differing from amino acids 49-55 by one amino acid; and a third V_l CDR comprising, consisting, or consisting essentially of amino acids 88-96 of SEQ ID NO: 21 or a sequence differing from amino acids 88-96 by one amino acid.

In additional embodiments: the V_h region is either SEQ ID NO: 20, a humanized SEQ ID NO: 20 , or a de-immunized SEQ ID NO: 20; and the V_l region is either SEQ ID NO: 21, a humanized SEQ ID NO: 21, or a de-immunized SEQ ID NO: 21.

In different embodiments focusing on an antibody heavy and/or light chain, the recombinant gene encodes either or both a protein comprising, consisting, or consisting essentially of: (a) a heavy chain comprising a V_h region as provided in Section III *supra*, a human hinge, CH_1, CH_2, and CH_3 from an IgG1, IgG2, IgG3 or IgG4 subtype or (b) a light chain comprising a V_l region as provided in Section III *supra*, and a human kappa C_L or lambda C_L. In a further embodiment the heavy chain consists essentially of the amino acid sequence of SEQ ID NO: 22; and the light chain consists essentially of the amino acid sequence of SEQ ID NO: 23.

V. Applications of Antigen Binding Proteins.

Antigens containing certain ORF0657n regions can be used to provide a protective immune response against *S. aureus* infection. (Anderson *et al.*, International Publication No. WO

2005/009379, International Publication Date February 3, 2005.) An antigen binding protein recognizing an ORF0657n target region can be used to facilitate the production, characterization, or study of ORF0657n antigens and vaccines. Antigen binding protein recognizing appropriate epitopes can also have therapeutic applications.

Examples of different uses in the production, characterization, or study of ORF0657n related antigens and vaccines include:

1) Identifying the presence of an ORF0657n antigen, for example, by Western blot;
2) Identifying the presence of an ORF0657n antigen on a cell surface, for example, by flow cytometry. This is useful, for example, in determining expression on multiple strains of *S. aureus* as well as confirmation of knock-out mutants;
3) Passive protection experiments. The antibodies can be used in a lethal model to determine if a specific area of the ORF0657n protein confers protection;
4) An immunoassay. The assay can be used to monitor antigen quality, product production and stability;
5) As a control in mouse potency assays to monitor immunogenicity of an antigen vaccine product; and
6) Serology assays can utilize a monoclonal antibody in a competitive format to identify an immune response to ORF0657n derived antigen vaccinated patients.

Techniques for using antigen binding proteins, such as monoclonal antibodies, in the production, characterization, or study of a target protein are well known in the art. (See, for example, Ausubel, *Current Protocols in Molecular Biology,* John Wiley, 2005, Harlow *et al., Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988, Harlow *et al., Using Antibodies,* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory Press, 1999, Lipman *et al., ILAR Journal 46*:258-268, 2005.)

In an embodiment of the present invention, the presence of an ORF0657n antigen in a solution, bound to a microsphere or on a cell is determined using an antigen binding protein. The ability of the binding protein to bind to a protein present in the solution or cell can be determined using different techniques such as a Western blot, enzyme-linked immunosorbent assay (ELISA), flow cytometry, and Luminex immunoassay.

VI. Treatment

Therapeutic and prophylactic treatment can be performed on a patient using an antigen binding protein binding to an appropriate target region. Therapeutic treatment is performed on those persons infected with *S. aureus.* Prophylactic treatment can be performed on the general population or a subset of the general population. A preferred subset of the general population are those persons at an increased risk of *S. aureus* infection.

A "patient" refers to a mammal capable of being infected with *S. aureus*. Preferably, the patient is a human. However, other types of mammals such as cows, pigs, sheep, goats, rabbits, horses, dogs, cats, monkeys, rats, and mice, can be infected with *S. aureus*. Treatment of non-human patients is useful in protecting pets and livestock, and in evaluating the efficacy of a particular treatment.

Persons with an increased risk of *S. aureus* infection include health care workers; hospital patients; patients with a weakened immune system; patients undergoing surgery; patients receiving foreign body implants, such as a catheter or a vascular device; patients facing therapy leading to a weakened immunity; and persons in professions having an increased risk of burn or wound injury. (*The Staphylococci in Human Disease*, Crossley and Archer (ed.), Churchill Livingstone Inc. 1997.)

In an embodiment, a patient is administered an antigen binding protein in conjunction with surgery or a foreign body implant. Reference to "surgery or a foreign body implant" includes surgery with or without providing a foreign implant, and providing a foreign implant with or without surgery. The timing of administration can be designed to achieve prophylactic treatment and/or therapeutic treatment. Administration is preferably started around the same time as surgery or implantation.

Guidelines for pharmaceutical administration in general are provided in, for example, *Remington's Pharmaceutical Sciences 20th Edition*, Ed. Gennaro, Mack Publishing, 2000; and *Modern Pharmaceutics 2nd Edition*, Eds. Banker and Rhodes, Marcel Dekker, Inc., 1990.

Pharmaceutically acceptable carriers facilitate storage or administration of an antigen binding protein. Substances used to stabilize protein solution formulations include carbohydrates, amino acids, and buffering salts. (Middaugh *et al.*, Handbook of Experimental Pharmacology *137*:33-58, 1999.)

Antigen binding proteins can be administered by different routes such as intraveneous, subcutaneous, intramuscular, or mucosal. Subcutaneous and intramuscular administration can be performed using, for example, needles or jet-injectors. Mucosal delivery, such as nasal delivery, can involve using enhancers or mucoadhesives to produce a longer retention time at adsorption sites. (Middaugh *et al.*, *Handbook of Experimental Pharmacology 137*:33-58, 1999.)

Suitable dosing regimens are preferably determined taking into account factors well known in the art including age, weight, sex and medical condition of the patient; the route of administration; the desired effect; and the particular compound employed. It is expected that an effective dose range should be about 0.1 mg/kg to 20 mg/kg, or 0.5 mg/kg to 5 mg/kg. The dosing frequency can vary depending upon the effectiveness and stability of the compound. Examples of dosing frequencies include biweekly, weekly, monthly and bimonthly.

VII. Examples

Examples are provided below further illustrating different features of the present invention. The examples also illustrate useful methodology for practicing the invention. These examples do not limit the claimed invention.

Example 1: Generation of Monoclonal Antibodies to ORF0657n

Monoclonal antibodies directed to ORF0657n (SEQ ID NO: 1) were generated using ORF0657n-C/e (SEQ ID NO: 2) or ORF0657n-H/y (SEQ ID NO: 3) as an antigen. The antibodies were identified and characterized by ELISA and flow cytometry.

Mice and Immunizations: Female BALB/c mice, 4-5 weeks old, were purchased from Taconic (Germantown, N. Y.). Mice were immunized intramuscularly (i.m.) on days 0, 7, and 21, with 20 μg of *E. coli* produced ORF0657n-C/e antigen or Yeast expressed ORF0657n-H/y antigen, formulated on aluminum hydroxyphosphate adjuvant. (Anderson *et al.*, International Publication No. WO 2005/009379, International Publication Date February 3, 2005.) A final intravenous injection (i.v.) of 20 μg of protein in phosphate buffered saline (PBS) was given to mice three days prior to the fusion. Mice were sacrificed and the spleens removed for cell fusion.

MAb Production: Lymphocytes prepared from spleens were fused with the mouse myeloma partner SP2/0-Ag14 (ATCC 1581) by polyethylene glycol 1500 (Boehringer Mannheim) at a ratio of 3:1. The fusions were plated into 96-well flat-bottomed microtiter plates in Dulbecco's Modification of Eagle's Medium, high glucose, pyruvate (DMEM) containing 20% fetal bovine serum, hypoxanthine (10^{-4} M), thymidine (10^{-5} M), Aminopterin (4×10^{-7} M) was added 24 hours later. Supernatants from growing hybridomas were screened by ELISA for reactivity to ORF0657n as described below. Positive wells were cloned by limiting dilution and retested for ELISA reactivity. Monoclonal antibodies were classified with an antibody-isotyping kit (Roche Diagnostics Corporation, Indianapolis, IN).

ELISA: Costar medium binding microtiter plates were coated overnight at 2-8°C with 50 nanograms per well of *E. coli* expressed SEQ ID NO: 2 in PBS. The plate was washed three times with PBS, 0.05% Tween20 and blocked with 1% Bovine serum albumin, PBS, 0.05% Tween20 (assay diluent) for at least 1 hour. The plate was washed as before and supernatants from the fusion wells or cloned hybridomas were added and allowed to incubate for 2 hours at room temperature. The plate was washed as before and a Goat anti-mouse IgG (H+L)-HRP conjugate (Zymed) (1:8000 in assay diluent) added and allowed to incubate for 1 hour at room temperature. Assay plates were developed with TMB substrate, the reaction stopped with 2.0 N H_2SO_4 and read in a plate reader at OD 450 nm. Wells were considered positive that had an optical density at 450 nm of >1.0.

Flow Cytometry: Prepared glycerol stocks of *S. aureus* passaged under iron-starved conditions (in RPMI) were used to evaluate mAb for ORF0657n binding. Frozen glycerol stock cells were thawed and resuspended in PBS; 1% bovine serum albumin; 0.1% sodium azide, 0.2% Pig IgG (Sigma) (PAAG) to a concentration of 5×10^7 CFU/50 ul. A 50 ul aliquot of the cells were placed in a 1.5 ml Eppendorf tube per reaction. Fifty microliters of the hybridoma culture were added to each reaction tube and incubated for 1 hour at room temperature. The cells were washed by adding 1 mL of phosphate buffered saline; 1% bovine serum albumin; 0.1% sodium azide (PAA) to the tube. The cells

were pelleted by centrifugation (5500 rpm, 5 minutes). The supernatant was removed and the cells were mixed with 100 µL of secondary antibody (FITC-labeled goat anti-mouse Ig (BD Pharmingen) diluted 1:100 in PAAG). Incubation was for 1 hour at room temperature in the dark. After incubation, 1 mL PAA was added to the reaction mixture, the cells were pelleted (5500 rpm, 5 minutes) and supernatant removed. The pellets were resuspended in 1 mL of PBS and transferred to 12 x 75 mm tubes for FAC analysis.

Tubes were run on a BD-FACSCalibur flow cytometer instrument gated for bacterial cells and measuring the amount of FITC associated with the cells. A standard antibody with known binding to the surface of *S. aureus* was run in every assay. A negative control was run as cells and the secondary conjugate alone. Hybridoma wells were considered positive if the geometric mean value was greater than 30.

Two separate fusions resulted in a panel of twelve monoclonal antibodies (mAb). All of the mAbs were reactive in ELISA (Table 2). Ten of the twelve mAbs bound to the surface of bacteria as evidenced by flow cytometry. All of the mAbs were positive by Western Blot analysis with the wild type protein.

Table 2

mAbs/cell lines Fusion #1	mAbs/cell lines Fusion #2
1) 2H2.B8 IgG1	
2) 8H6.E11.H3 IgG2a*	
3) 7H2.C11 IgG1*	
	4) 2E12.A8 IgG1
	5) 8A8.B4 IgG1
	6) 3G11.D5 IgG1
	7) 13G11.C11 IgG1
	8) 13C7.D12 IgG1
	9) 1G3.B3 IgG1
	10) 9H3.E4 IgG1
	11) 3B7.G8 IgG1
	12) 3G12.A4 IgG1

* Not reactive in flow cytometry. Fusion #1 was generated from *E. coli* produced ORF0657n-C/e antigen. Fusion #2 was generated with Yeast expressed ORF0657n-H/y antigen.

Example 2: Class Switching mAbs

All of the mAbs isolated that bound to the native antigen were of the IgG1 isotype. These antibodies were class switched to an IgG2b isotype by selecting for shift variants (Spira *et al, J. of Immunogical Methods, 74*:307-315, 1985). A suitable immunoassay was developed using an IgG2b conjugate and the cell line was plated at a high density. Somatic cell mutations were selected, enriched

and then cloned. The binding site of the switched mAb remained identical to the original mAb, but switching to an IgG2b subtype gave a more favorable isotype (initiating the complement cascade) in the passive protection studies.

Table 3 Class Switched mAbs

IgG1 isotype	IgG2b isotype
2H2.B8	2H2.BE11
2E12.A8	2E12.BG1
8A8.B4	8A8.BF9
3G11.D5	3G11.BE5
13G11.C11	13G11.BF3
13C7.D12	13C7.BC1
1G3.B3	1G3.BD4
9H3.E4	9H3.BE4

Example 3: Binding Inhibition Studies with Native Antigen

Purified antibodies were labeled with Alexafluor-488 using a mAb labeling kit (Molecular Probes) according to the manufacturer's instructions. The amount of mAb that would just saturate the surface of RPMI-grown bacterial cells was determined for both the labeled and unlabeled mAbs. Each of the mAbs in Table 3 (1st column) were used labeled and unlabeled.

The inhibition assay was performed by first incubating 5 X 10^7 cells with the unlabeled mAb at a concentration that would saturate the surface of the cells. This reaction was incubated at room temperature for 1 hour. After this incubation, the reactions were washed with 1 ml of PAA and spun at 6,000 RPM for 5 minutes in a microcentrifuge (Hermle). The supernatant was removed down to ~50 ul and the cells were resuspended in 100 ul of PAAG containing the amount of directly labeled mAb that would just saturate the surface of the cells. After this incubation, the reactions were washed with 1 ml of PAA and spun at 6,000 RPM for 5 minutes in a microcentrifuge (Hermle). The supernatant was removed down to ~50 ul and the cells were resuspended in 1 ml of PBS and transferred to 12 x 75 mm tubes for FAC analysis. As controls, separate reactions with the unlabeled mAb were measured with a secondary Alexafluor-488 conjugated goat anti-mouse IgG (H+L) (Molecular probes, 1:400 in PAAG) to determine that this mAb was bound to the surface. A positive control was also performed that had only the labeled mAb with the cells. If the unlabeled mAb bound to the same epitope as the labeled mAb then there would be no or low fluorescent reactivity associated with the cells. If the unlabeled mAb bound to a different epitope than the labeled mAb then the level of reactivity associated with the surface would be equivalent to the labeled mAb only control cells.

The panel of monoclonal antibodies fell into four reactive groups by inhibition studies:

Table 4

Group I	Group II	Group III	Group IV
2H2.B8	9H3.E4	13G11.C11	2E12.A8
8A8.B4	1G3.B3		13C7.D12
	3G11.D5		

Example 4: Binding Studies with Denatured Antigen and Altered Antigens

ORF0657n altered proteins were used to further characterize binding. Nucleic acid encoding ORF0657n was initially cloned into the expression vector pET-28a (Novagen) and expressed in *E. coli* with a C-terminal 6X his tag (SEQ ID NO: 2). The expression vector with the cloned gene was subjected to mutagenesis using Stratagene's QuikChange XL Site-Directed Mutagenesis Kit following the manufacturer's instructions. The gene was mutated with specific sequential amino acid changes. The resulting plasmid was transformed into Stratagene's XL10-Gold competent cells following the manufacturer's protocol. Plasmids were isolated from transformants using Qiagen's QIAprep Spin Miniprep Kit. Transformants were screened by sequencing using ABI's 310 DNA Sequencer. Plasmid from the transformant exhibiting the greatest number of base changes was transformed into the expression host HMS174(DE3) (Novagen). Transformants were expressed following Novagen's instructions.

Different ORF0657n altered proteins were used to determine the diversity of the ORF0657n mAbs (SEQ IDs 4-19). These proteins were screened with the 10 different mAbs in dot blots using standard procedures. Positive/negatives were confirmed by Western blots using standard procedures. By this approach antibodies were grouped according to their binding profile. Seven of the antibodies resolved to three groups; the three remaining antibodies (2H2.B8, 8A8.E11.H3 and 13G11.C11) had profiles that were similar but not identical to each other (Table 5).

TABLE 5: Binding of ORF0657n specific mAbs to ORF0657n mutant proteins detected by Western blot

	Group III		Group II			Group IV		Group I		
SEQ ID NO:	3G11.C11	3G12.A4	3B7.G8	1G3.B3	9H3.E4	2E12.A8	13C7.D12	2H2.B8	8A8.E11.H3	13G11.C11
1	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+

TABLE 5: Binding of ORF0657n specific mAbs to ORF0657n mutant proteins detected by Western blot

	Group III		Group II			Group IV		Group I		
SEQ ID NO:	3G11.C11	3G12.A4	3B7.G8	1G3.B3	9H3.E4	2E12.A8	13C7.D12	2H2.B8	8A8.E11.H3	13G11.C11
4	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	-	-	+	+	+
7	+	+	+	+	+	-	-	+	+	+
8	+	+	+	+	+	-	-	+	+	+
9	+	+	+	+	+	-	-	-	+	+
10	+	+	+	+	+	-	-	-	+	+
11	-	-	W	W	W	-	-	-	-	W
12	-	-	W	W	W	-	-	-	-	W
13	-	-	-	-	-	-	-	-	-	-
14	+	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	-	+	+
17	+	+	+	+	+	+	+	-	+	+
18	+	+	+	+	+	+	+	-	+	-
19	+	+	+	+	+	W	W	-	-	+

+, Antibody bound to protein in a Western; -, Antibody did not bind to protein by Western; W, Weak binding of antibody to protein detected by Western. Antibodies were grouped according to hybridization profile. A dotted line is used where similar, but not identical profiles were obtained.

Example 5: BAIcore Studies

In BIAcore studies the mAbs were examined by "footprint analysis" using purified ORF0657n-H/y as the antigen. Pair-wise binding experiments were conducted using real-time biomolecular interaction analysis via BIACORE ®. BIACORE ® incorporates microfluidics technology and surface plasmon resonance (SPR) to detect changes in mass by monitoring changes in the refractive index of a polarized light aimed directly at the surface of a carboxyl methyl dextran coated (CM5) sensor chip. The changes in response, measured in Response Units, can be correlated to the amount of bound analyte (*i.e.* antigen or antibody).

An anti-staphylococcal antibody (mAb 13C7.D12) was covalently bound (immobilized) on the surface of the CM5 sensor chip. The immobilized Ab was exposed first to the ORF0657n protein and subsequently to a pair of antibodies in a matrix format. After each cycle of ORF0657n protein + antibody pair, the surface of the sensor chip was regenerated back to the immobilized mAb 13C7.D12

using 20 mM HCl. Eight antibodies were tested against the ORF0657n protein in a matrix format so that all combinations of each antibody pair could be analyzed. The matrix design for mAb pairs used in this experiment is summarized in Table 6.

Table 6. Summary of Antibodies Tested in 8x8 Matrix

		Second Antibody			
Cycle #	First Antibody	Flow Cell 1	Flow Cell 2	Flow Cell 3	Flow Cell 4
1	N/A Immobilization	13C7.D12	13C7.D12	13C7.D12	13C7.D12
2	2H2.B8	2H2.B8	13C7.D12	8A8.B4	9H3.E4
3	2H2.B8	13G11.C11	2E12.A8	1G3.B3	3G11.D5
4	13C7.D12	2H.B82	13C7.D12	8A8.B4	9H3.E4
5	13C7.D12	13G11.C11	2E12.A8	1G3.B3	3G11.D5
6	8A8.B4	2H2.B8	13C7.D12	8A8.B4	9H3.E4
7	8A8.B4	13G11.C11	2E12.A8	1G3.B3	3G11.D5
8	9H3.E4	2H2.B8	13C7.D12	8A8.B4	9H3.E4
9	9H3.E4	13G11.C11	2E12.A8	1G3.B3	3G11.D5
10	13G11.C11	2H2.B8	13C7.D12	8A8.B4	9H3.E4
11	13G11.C11	13G11.C11	2E12.A8	1G3.B3	3G11.D5
12	2E12.A8	2H2.B8	13C7.D12	8A8.B4	9H3.E4
13	2E12.A8	13G11.C11	2E12.A8	1G3B3	3G11.D5
14	1G3.B3	2H2.B8	13C7.D12	8A8.B4	9H3.E4
15	1G3.B3	13G11.C11	2E12.A8	1G3.B3	3G11.D5
16	3G11.D5	2H2.B8	13C7.D12	8A8.B4	9H3.E4
17	3G11.D5	13G11.C11	2E12.A8	1G3.B3	3G11.D5

To normalize for the amount of antigen initially bound (captured) in each run, the following ratio for each test antibody/antigen complex is calculated:

$$= \frac{\text{Test Antibody Response Units* 1000}}{\text{ORF0657n protein Response Units}} \quad \text{or} \quad \frac{mRU_{Ab}}{RU_{Ag}}$$

The percentage of available epitope remaining for each antibody can be calculated for the mapping pair as follows:

$$= \frac{(mRU_{Ab} \text{ (when } 2^{nd} \text{ Ab)} / RU_{Ag})*100}{(mRU_{Ab} \text{ (when } 1^{st} \text{ Ab)} / RU_{Ag})} \quad \text{or} \quad \text{\% Remaining (calculated for each Ab)}$$

Figure 2 illustrates matrix resulting outlining the reactivities of the monoclonal antibodies in a pair-wise binding study. The panel of monoclonal antibodies fell into three reactive areas by the BIACORE® method (See Table 7).

Table 7

Group I	Group II	Group III
2H2.B8	13G11.C11	13C7.D12
8A8.B4	3G11.D5	2E12.A8
9H3.E4		
1G3.B3		

Example 6: Protection Studies with Passive Immunization in a Murine Sepsis Model

The monoclonal antibodies mAb 2H2.BE11 and mAb 13C7.BC1 were tested for their ability to provide protection against *S. aureus* infection. These antibodies recognize different epitopes on the ORF0657n protein. Controls included an isotype matched mAb and PBS-only.

The mAbs or PBS were administered intraperitoneally (i.p.) 20 hours prior to bacterial challenge. Mice were then challenged with a LD_{80-90} dose of *S. aureus* Becker i.v. and monitored for survival. Each experiment was repeated three times with groups of 10 or 20 mice and was monitored for 10 days. The half life for the monoclonal antibodies in uninfected BALB/c mice is approximately eight days. A dose of 0.5 mg was found to be optimal. The results of experiments with the two monoclonal antibodies are presented in Figures 3A-C, 4A, 4B, and 5A-C.

Whereas the mAb 13C7.BC1 significantly improved survival at day 10 compared to the controls in one experiment, in the other 2 repetitions the overall survival rate was similar to that of the controls (Figures 3A-3C). However, compared to controls, there was delay in the time to death of the mAb 13C7.BC1 treated mice within this 10 day period. A similar trend in delay of time to death of the mAb 2H2.BE11 treated mice was also noted in two of the three experiments (Figures 5A-5C).

The effect of mAb 13C7.BC1 was also examined using a recent *S. aureus* clinical isolate UK58 (Figures 4A and 4B). This strain was minimally passaged from an abscess site in a patient. In two independent experiments, the results show a delay in time to death with the UK58 challenge.

Antibody persistence studies cannot be evaluated in the LD_{80-90} model due to the rapid rate of death. Therefore, a sub-lethal challenge model was run. In the sub-lethal model the challenge dose used is 10% of that used for the LD_{80-90} model. The sub-lethal challenge model was monitored over a four day period. Groups of 22 mice received 0.5 mg doses of either mAb 13C7.BC1 or isotype control mAb (6G6) 20 hours prior to i.v. bacterial challenge with 5×10^7 CFU of *S. aureus* Becker. Two animals from each group were sacrificed just prior to challenge (T=0) to determine the mAb levels in the

serum at the time of challenge. At 2, 24, 48, 72 and 96 hours post challenge, four mice from each group were sacrificed and serum mAb levels determined.

From this sub-lethal challenge experiment, the half life of mAb 13C7.BC1 in *S. aureus*-infected mice was estimated to be approximately one-day. In contrast, the half life of the isotype control mAb was estimated to be greater than four days (data not shown). These data point to a specific reduction of mAb 13C7.BC1 in *S. aureus* challenged mice, which appears to be exhausted well before the ten day period monitored in the lethal model.

In six of the eight experiments illustrated in Figures 3A-C, 4A, 4B, and 5A-C, improved survival was observed through approximately three days for the groups receiving the mAb administration. These results provide an indication that such mAbs have a positive effect on the survival rate of *S. aureus* challenged mice.

Example 7: Protection Studies with Passive Immunization in a Murine Indwelling Catheter Model

A murine indwelling catheter model was used with mAb 2H2.BE11. The *S. aureus* strain used in this model was the clinical isolate MCL8538. This strain was selected as lower inocula could be administered while still getting reproducible colonization of catheters compared to *S. aureus* Becker, the strain used in the murine sepsis model.

ICR mice had catheters (PE50 silicone rubber) surgically implanted into the jugular vein, held in place with sutures, and exiting with a port on the dorsal midline of the mouse. Mice were rested 9-11 days post surgery. At 24 hours prior to challenge, mice were passively immunized with a single injection of 600 mcg of murine monoclonal antibody 2H2.BE11 administered i.p. At day 0, mice were challenged with *S. aureus* MCL8538 administered i.v. The inoculum dose was 2 -8 X 10^5 CFU in 100 µl volume (Experiments 1 to 3). This low dose was found to clear spontaneously from the catheters after 4 days. Therefore, catheters were assessed for bacteria at 24 hours post challenge. At that time, mice were sacrificed and catheters harvested. The presence of bacteria on the catheters was assessed by culturing the entire catheter on TSA. If any sign of outgrowth was observed on the plate the catheter was scored as culture positive.

In two of the first three experiments, the number of culture negative catheters was significantly lower in mice passively immunized with antibody 2H2.BE11, as compared to the isotype control antibody. A fourth experiment was performed using a larger inoculum dose. In this more rigorous challenge, the dose was determined to be one in which 100% of catheters were reproducibly infected, and this infection was not spontaneously cleared by control mice (monitored over 7 days). In experiment 4, with the larger inoculum size, again, significantly fewer catheters were found to be infected in mice injected with antibody to 2H2.BE11, compared with the isotype control. Results of the four experiments are summarized in Table 8.

Table 8: Number Of Culture Negative Catheters Obtained In 4 Independent Passive Transfer Experiments Using a Murine Indwelling Catheter Model

	Number of Culture-Negative Catheters					p-value
Monoclonal	Exp#1	Exp#2	Exp#3	Exp#4	Total	
2H2.BE11	3 of 4 (75%)	6 of 8 (75%)	4 of 10 (40%)	4 of 9 (44%)	17/31 (54%)	0.0187
Isotype matched control	1 of 4 (25%)	3 of 8 (38%)	4 of 10 (40%)	0 of 9 (0%)	8/31 (25%)	

Groups of ICR mice with indwelling catheters were injected i.p. with 600 mcg of murine monoclonal antibody 24 hours prior to challenge, all monoclonals of the IgG2b isotype

Example 8: Ex-Vivo Pre-Opsonization of Bacteria Using anti-ORF0657n Monoclonal Antibodies 2H2.B8 (IgG1), 2H2.BE11 (IgG2b), or 13C7.IgG2b or Isotype Matched Control mAbs

To test whether monoclonal antibodies to ORF0657n are opsonic, passive protection experiments were conducted in which a lethal dose of *S. aureus* was pre-opsonized with the monoclonal antibodies 2H2.B8, 2H2.BE11, or 13C7.IgG2b, or an isotype matched control monoclonal antibody. Pre-opsonized bacteria were then administered to mice i.p. Bacteria used in these experiments were *S. aureus* RN4220 (wild type) or RN4220.0657n. The RN4220.0657n bacteria were engineered to express ORF0657n in the absence of control by the FUR box. Therefore, they could be grown in the presence of iron and still express ORF0657n antigen on their surface. Alternatively, RN4220 (wild type) was passed 2X in a low iron medium RPMI to induce expression of 0657n on the bacteria surface.

A quantity of bacteria sufficient for 6 Balb/c mice (6 X LD_{100}) was incubated with 800 µg IgG at 4 °C for 1 hour, with gentle rocking. Bacteria were then pelleted and any unbound mAb removed. Antibody-opsonized bacteria were re-suspended in 2.4 mL of PBS, and 0.4 mL (1 X LD_{100}) was injected into each of five mice. After challenge, each inoculum was quantitated by plating on TSA to insure that equivalent CFU was given to all groups of mice and that the mAbs had not aggregated the bacteria. Survival was monitored for 3 days post challenge. Since the target antigen must be present on the surface of the bacteria for this procedure to be effective, care was taken to ensure that 0657n was expressed on the bacteria prior to opsonization. ORF0657n expression was monitored by flow cytometry using mAb 2H2.B8. The dose of opsonized bacteria injected into each mouse was 2-4 X 10^9 CFU RN4220.0657n/mouse, or 1-2 X 10^9 CFU RN4220(2X RPMI)/mouse.

When pre-opsonized with either 2H2.B8 or 2H2.BE11, but not an isotype matched control mAb, mice were protected from death from a lethal dose of RN4220.0657n staphylococci. The experiment was repeated twice for the IgG1 isotype and three times for the IgG2b isotype with similar results (Table 9A).

Table 9A: Ex-vivo Protection with Anti-0657n mAb

Monoclonal	Exp 1 Surviving Mice	Exp 2 Surviving Mice	Exp 3 Surviving Mice	Total
2H2.BE11 (IgG2b)	5	4	5	93% (14/15)
6G6.A8 (IgG2b)	1	0	1	13% (2/15)
PBS	1	2	0	20% (3/15)
2H2.BE11 (IgG1)	ND	4	5	90% (9/10)
10B4.H4 (IgG1)	ND	1	1	20% (2/10)

Five mice were used in each experiment. Challenge strain RN4220.0657n.pYZ119. Dose: 2-4 X 10^9 CFU. Test mAbs: murine anti-0657n 2H2.BE11 (IgG2b); 2H2.B8 (IgG1).

When pre-opsonized with either mAb 2H2.B8 but not an isotype matched control mAb, mice were protected from death from a lethal dose of RN4220 (2X RPMI) staphylococci. The experiment was repeated six times with similar results (Table 9B).

Table 9B: Ex-vivo Protection with Anti-0657n mAb

Monoclonal	# Tests	Aggregate	% Survival
2H2.B8	6	30/30	100%
10B4.IgG1 Isotype control	6	2/30	7%
13C7.IgG2b	2	0/10	0%
6G6.IgG2b Isotype control	2	0/10	0%

Murine anti-0657n 2H2 was very effective in preventing death in this lethal model. The 13C7 mAb was not effective in this model (as opposed to the previously described model illustrated in Figures 3-6). All (2H2.BE11, 2H2.B8 and 13C7.IgG2b) of the anti-0657n mAb's bind RN4220 (as demonstrated using flow cytometery) and all have opsonizing activity in the *in vitro* OPA assay. This model reflects an additional requirement for epitope specificity for enhancing survival in the peritoneum of the mouse.

Example 8: Epitope mapping studies performed with 2H2 mAb

The experiments described in this example provide evidence that the monoclonal antibody 2H2.BE11 recognizes a conformational epitope within ORFO657n. The experiments localized the minimal sequence within ORFO657n required for displaying the conformational epitope in a three dimensional structure recognized by 2H2 mAb. In addition, the experiments identified distinct lysine

residues within the minimal sequence of ORFO657n that become protected from reacting with small molecules when 2H2 mAb is bound to ORFO657n.

The potential ability of 2H2 mAb to recognize linear epitopes of typically 9 to 14 amino acids in length within the sequence of ORFO657n was investigated using epitope extraction and starting with an ORF0657n fragment from amino acid 42 to amino acid 486 of SEQ ID NO: 1 ("ORF0657t"). In detail: 30 ug of 2H2 mAb were immobilized by chemical cross linking to 10 mg of cyanogen bromide activated sepharose (Amersham cat. No. 17 0430 01) for each of the epitope extraction experiments. Proteolytic digests of the ORF0657t were generated with GluC (Roche Applied Science cat. No. 11 420 3997 001), Asp-N (Roche Applied Science cat. No. 11 054 589 001) or Chymotrypsin (Roche Applied Science cat. No. 11 418 467 001) and characterized by 1D/LC-MS/MS on a linear ion trap (LTQ – Thermo Electron Inc). In three individual experiments 8.4 µg of the characterized proteolytic digest from any protease was allowed to react with the immobilized antibody. Unbound peptides were washed off the antibody cross-linked beads. Potentially bound peptides were eluted with low pH and characterized by 1D/LC-MS/MS. None of the generated proteolytic peptides were recognized with high efficiency and specificity by 2H2 mAb, providing a strong indication that 2H2 mAb did not recognize a linear epitope.

The finding that 2H2 mAb did not recognize a linear sequence of ORFO657n was corroborated by a limited chemical cleavage experiment. ORF0657t was chemically cleft with CNBr for 2 hours. The resulting cleavage products were analyzed by SDS-PAGE. SDS-PAGE analysis showed 5 major bands with molecular weights of approximately 42 kDa, 35 kDa, 25 kDa, 15 kDa and 10 kDa. A Western Blot analysis with 2H2 mAb clearly showed that only the 42 kDa band was recognized by 2H2. All bands were excised from the SDS-PAGE, in-gel digest was performed, and the resulting peptides that were identified by tandem mass spectrometry were matched to corresponding sequences in ORF0657t. The result of the analysis of the major bands is shown in Table 10:

Table 10

CNBr cleavage	Binds to 2H2 mAb	ORFO657t	Calculated MW kDa
Band 42 kDa	yes	[001-356]	40.7
Band 35 kDa	no	[001-323]	36.7
Band 25 kDa	no	[001-214]	23.9
		[116-302]	21.9
Band 15 kDa	no	[215-356]	16.8
		[303-446]	16.6
Band 10 kDa	no	[114-214]	11.7
		[215-302]	10.39
		[357-446]	10.28

The importance of a fragment with a molecular weight of ~ 42 kDa was confirmed by epitope excision. In detail, 210 µg of 2H2 mAb was immobilized by chemical cross linking to 50 mg of cyanogen bromide activated sepharose (Amersham cat. No. 17 0430 01) for each of the epitope excision experiments. Then, 50 µg of intact ORF0657t was allowed to bind to the immobilized antibody and non-bound ORF0657t washed off by intensive washing with phosphate buffered saline. In three independent experiments proteases Glu-C, Trypsin and a sequential combination of GluC, AspN, Trypsin, Chymotrypsin, and Carboxy-peptidase Y were added for 5 hours or one hour per protease in the sequential combination. Peptides that were excised by the proteases during the incubation were thoroughly washed away and ORF0657t fragments that specifically bound to 2H2 mAb released with SDS loading buffer.

Fragments that specifically bound to 2H2 mAb were analyzed by SDS-page. All three of the epitope excision experiments showed exclusively one band with a molecular weight between 40 and 42 kDa in the SDS-Page analysis. Bands binding to 2H2 mAb were confirmed by Western Blot analysis. The epitope excision experiment was repeated for the Glu-C protease. This time the fragment of ORF0657t that specifically bound to 2H2 mAb was released with acidic conditions and analyzed by 1D/LC-MS/MS on a linear ion trap (LTQ, Thermo Electron). The eluted sample showed a signal (total ion count) with the expected intensity at 82-87 minutes (40%– 45% acetonitrile) and multiple charge states ($[M+67\ H]^{67+}$ to $([M+30\ H]^{30+}$) that deconvoluted to 42.628 kDa. A possible fragment of ORFO657t corresponding to this particular mass is sequence [012-382] of ORFO657t with a molecular weight of 42.6 kDa.

To determine which lysine residues of ORFO657t are protected from chemical reactions upon binding of 2H2 mAb, chemical labeling experiments were preformed with sulfo-NHS-acetate (Pierce Cat. No. 26777) using three different experimental conditions in the presence or absence of 2H2 mAb. See Table 11.

Table 11

Experiment	1	2	3
molar excess 2H2 mAb	0 or 3	0 or 3	0 or 3
molar excess sulfo-NHS acetate	25	500	75
Reaction temperature °C	room temperature	15	37
Reaction time	1 hour	30 minutes	2 hours

For each experiment, reaction products produced with O or 3 molar excess 2H2 mAb were incubated with one of three proteases resulting in 2 x 9 reaction mixtures. Experiment 1 employed

GluC, AspN and Trypsin. Experiments 2 and 3 employed GluC, AspN, and Chymotrypsin. The proteolytic peptides were then analyzed by 1D/LC-MS/MS. For each of the reactions a ratio of acetylated and non-acetylated lysine residues was calculated based on the area under curve of the total ion count (TIC) of the individual peptides. Obtained ratios were then compared between the pairs (with and without 2H2 mAb) for identical reaction conditions. A global analysis was performed for all three reaction conditions to identify lysine residues within ORF0657t that are maximally shielded upon binding of ORF0657t to 2H2 mAb. The chemical labeling experiment described above identified K76, K257 and potentially K443 as being most protected upon binding of 2H2 mAb. Protection against chemical labeling is likely due to direct binding. However, it is possible that such protection could be due to binding in close proximity to the protected sites or by long range structural changes within ORFO657nI

In summary, the above described experiments provide clear evidence that the epitope within ORF0657t that is recognized by the 2H2 mAb is conformational. The fragment of ORF0657t that is recognized by 2H2 mAb has an N-terminus located between amino acids 1 and 115 of ORF0675t and a carboxyl terminus located between amino acids 323-357 of ORF0657t. Even though it can not be excluded that protection from chemical labeling upon binding of 2H2 mAb is influenced by long range structural changes, it is very likely that areas in close proximity to Lysine 76 and Lysine 275 participate in direct antibody interaction.

Example 9: 2H2 mAb Sequence Identification

Identification of the variable light (V_l) and variable heavy (V_h) sequences of hybridoma expressed 2H2 IgG was accomplished by combining the degenerative primer PCR /overlap extension cloning process for single chain variable fragments (scFv) assembly (Krebber *et al. JIM 201(1)*:35-55, 1997), with high throughput screening of soluble scFv fused to a human kappa light chain constant domain or scAb material via Biacore. This allowed for fine discrimination of mutations in V_l frameworks 1, 4 and V_h frameworks 1, 4 generated by the degenerative primer method.

Briefly, RNA material was purified from the hybridoma cell line using standard methods from a Total RNA Kit™ (Ambion Inc.). This material was then reverse transcribed to cDNA and utilized as template in PCR to amplify the variable regions. The conditions for the PCR amplification of the V_l and V_h chains was based upon the protocol described by Krebber *et al. JIM 201(1)*:35-55, 1997. The primers are designed such that a $(Gly4Ser)_4$ linker (SEQ ID NO: 32) is added which provides domains for a third PCR reaction in which the V_h and V_l are overlapped to create a V_l-$(Gly4Ser)_4$-V_h scFv.

The first set of PCR reactions to amplify the variable chains individually, were carried out in a volume of 100 µl containing 5 µl of the cDNA reaction, 2 µM each of the forward and reverse primer sets for amplification of V_l and V_h, and a high fidelity PCR master mix. The reactions were denatured for 4 minutes at 94°C followed by 30 cycles of 30 seconds at 94°C, 30 seconds at 50°C, 1

minute at 72°C, and finished at a final cycle of 5 minutes at 72°C. The full length PCR products were gel purified.

To construct the full length product a third PCR reaction was done to assemble to scFv from the amplified V_h and V_l material. In a volume of 100 µl approximately 20 ng each of V_h and V_l DNA and a high fidelity PCR master mix was denatured for 5 minutes at 94°C, followed by 3 cycles of 30 seconds at 94°C, 30 s at 60°C, and 30 seconds at 72°C in the absence of primers. The modified PCR primers, SEQ ID NO: 33 and SEQ ID NO: 34 were added at a final concentration of 1 µM, and 30 cycles of 30 seconds at 94°C, 1 minutes at 60°C, and 1 minute at 72°C were performed, followed by 7 minutes at 72°C. The expected full length scFv PCR products were gel purified.

The amplified scFv material was cloned into the MP16 soluble expression vector for scAb production (Hayhurst *et al.*, *JIM 276(1-2)*:185-196, 2003) and sequence analysis. A single restriction enzyme digest with Sfi1 was used for directional cloning into the MP16 vector. Clones with apparent full length variable heavy and variable light chains present were then expressed as scAbs in XL1-Blue cells and recovered from the periplasm using a standard osmotic shock procedure. Briefly, clones were grown at 37°C overnight in growth media containing 2% glucose and 100 µg/ml ampicillin in a 96 well format. 20 µl of the overnight culture was transferred to new media containing 0.1% glucose and 100 µg/ml ampicillin and grown until an OD_{600} of 0.6 was reached. The cells were induced for scAb expression by adding IPTG at a final concentration of 0.5 mM and incubated overnight while shaking at 150 rpm, at room temperature. The scAbs were purified from the cells using a Qiagen Ni-NTA superflow robotic procedure.

To analyze each scAb periplasmic preparation for binding activity to ORF0657t, a Biacore3000 surface plasmon resonance (SPR) instrument (Upsala, Sweden) was utilized. Standard EDC/NHS coupling was used to covalently mobilize approximately 250 resonance units of the 0657t antigen directly to the experimental flow cell surface of a CM5 sensor chip. A reference flow cell surface was activated and deactivated without coupling of protein. Each preparation was then run over the surface and association and dissociation of the scAb to antigen was measured. The surfaces were regenerated between runs by a single injection of 10 mM HCl for 20 seconds at a flow rate of 20 µl/min, followed by a 2 minute stabilization period. All samples were run in duplicate and buffer only runs were used as controls. After screening 95 clones, a clone was selected based on its binding activity. The final 2H2 clone chosen was based upon its similar affinity for ORF0657t as the original hybridoma prepared IgG material as well as comparative sequence analysis.

The amino sequence of the 2H2 V_h (SEQ ID NO: 20) and V_l (SEQ ID NO: 21) were as follows:

2H2 V_h Amino Acid Sequence (SEQ ID NO: 20)

```
  1   DVHLVESGPG LVAPSQNLSI TCTVSGFSLS RYGVHWVRQP PGKGLEWLGL
 51   IWAGGVTIYN STLMSRLSIS KDSSKSQVFL KMNSLQIDDT AIYYCAREAS
101   RDHYFDYWGQ GTTLTVSS
```

2H2 V_l Amino Acid Sequence (SEQ ID NO: 21)

```
  1   DIVMTQSPAI MSASPGEKIT MTCSASSSVS YIYWYQQKSG TSPKRWIYDT
 51   SKLASGVPFR FSGGGSGTSF SLTISSMEAE DAATYYCQQW SSNPLTFGAG
101   TKLEIK
```

The underlined portions are the CDR's. CDR's were identified based on the Kabat definition. The encoding nucleic acid sequence is provided by SEQ ID NO: 24 (V_h) and SEQ ID NO: 25 (V_l).

Example 10: *2H2 IgG Chimera Expression*

The variable regions for 2H2 mAb were cloned from mouse hybridoma as described in Example 9. The sequences for the variable regions were PCR amplified and DNA encoding the heavy chain variable regions were fused in-frame with DNA encoding the IgG1 constant region whereas DNA encoding the light chain variable region were fused in-frame with DNA encoding the kappa constant region. The cloning procedure for the resulting antibody expression vectors is described below.

The variable regions were PCR amplified. PCR reactions were carried out in a volume of 25 µl containing high fidelity PCR master mix, template volume 1 µl and forward and reverse primers: 1 µl each. PCR condition was 1 cycle of 94°C, 2 minutes, 25 cycles of 94°C, 1.5 minutes; 60°C, 1.5 minutes; 72°C, 1.5 minutes and 72°C, 7 minutes; 4°C until removed and cloned in-frame with leader sequence at the 5'-end and constant region at the 3'-end using In-Fusion strategy. The following primers were used: Light chain forward, 5'- ACAGATGCCAGATGCGATATTGTGATGACCCAGTCT (SEQ ID NO: 28); Light chain reverse, 5'- TGCAGCCACCGTACGTTTTATTTCCAGCTTGGTCCC (SEQ ID NO: 29); Heavy chain forward, 5'- ACAGGTGTCCACTCGGATGTGCACCTGGTGGAGTCA (SEQ ID NO: 30); and Heavy chain reverse, 5'- GCCCTTGGTGGATGCCGAGGAGACTGTGAGAGTGGT (SEQ ID NO: 31). The DNA sequences for all the clones were confirmed by sequencing.

The amino acid sequences deduced from DNA sequences are:

Mouse 2H2 Variable and Human Kappa Constant Region Amino Acid Sequence (SEQ ID NO: 22)

```
  1   DIVMTQSPAI MSASPGEKIT MTCSASSSVS YIYWYQQKSG TSPKRWIYDT
 51   SKLASGVPFR FSGGGSGTSF SLTISSMEAE DAATYYCQQW SSNPLTFGAG
101   TKLEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD
151   NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL
201   SSPVTKSFNR GEC
```

Mouse 2H2 Variable and Human IgG1 Constant Region Amino Acid Sequence (SEQ ID NO: 23)

1 DVHLVESGPG LVAPSQNLSI TCTVSGFSLS RYGVHWVRQP PGKGLEWLGL
51 IWAGGVTIYN STLMSRLSIS KDSSKSQVFL KMNSLQIDDT AIYYCAREAS
101 RDHYFDYWGQ GTTLTVSSAS TKGPSVFPLA PSSKSTSGGT AALGCLVKDY
151 FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTQTYI
201 CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAPELLGGPS VFLFPPKPKD
251 TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
301 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY
351 TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD
401 SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK

The variable regions are underlined.

The antibodies were expressed in 293EBNA monolayer cells. The plasmids were transfected using PEI based transfection reagents. The transfected cells were incubated in Opti-MEM serum free medium and the secreted antibodies were purified from medium using protein A/G affinity chromatography. The concentration of purified antibodies was determined by OD280 nm and the purity was measured by LabChip™ capillary electrophoresis.

The expression of both light and heavy chains was driven by human CMV promoter and bovine growth hormone polyadenylation signal. (Shiver *et al., Ann. N.Y. Acad. Sci., 772*:198-208, 1995.) The leader sequence in the front mediated the secretion of antibodies into the culture medium. The leader sequence for the heavy chain was MEWSWVFLFFLSVTTGVHS (SEQ ID NO: 26) and for the light chain was MSVPTQVLGLLLLWLTDARC (SEQ ID NO: 27). The expression vectors carry oriP from EBV viral genome for prolonged expression in 293EBNA cells and the bacterial sequences for kanamycin selection marker and replication origin in *E. coli*.

The antibodies were expressed in 293EBNA monolayer cells. The plasmids were transfected using PEI based transfection reagents. The transfected cells were incubated in Opti-MEM serum free medium and the secreted antibodies were purified from medium using protein A/G affinity chromatography. The concentration of purified antibodies was determined by OD280nm and the purity by LabChip capillary electrophoresis.

Example 11: Affinity Determination

Comparative analysis was performed on 2H2 mAb as hybridoma material, scAb and a chimeric antibody. 2H2 mAb V_h and V_l region were cloned and expressed as an IgG chimera as described in Example 10. scAb was cloned into the MP16 vector (Example 9), which produces a scFv with a Human Kappa chain tag fused to it. As further described below, the antigen affinity was not significantly different among the constructs.

To measure a 1:1 interaction between the binding domain and the antigen, the experimental set up on Biacore was modified depending on whether antibody fragment or full length IgG

was analyzed. For IgG measurements, the IgG was captured to the surface as ligand and ORF0657t was run as analyte. For antibody fragment analysis, ORF0657t was bound to the surface and the antibody fragment was run as the analyte. This demonstrated that the affinity of the original 2H2 mAb hybridoma material to the ORF0657t antigen shows no significant change upon recombinant cloning (Table 12). Data were acquired via surface plasmon resonance on a Biacore 3000; each analyte was run at multiple concentrations, with two replicates per concentration. Data were analyzed with BIAevaluation (Biacore, Inc.) with simultaneous fits of entire concentration series. Fit parameters are listed in Table 12.

Table 12

	On-rate ka (1/Ms)	Off-rate kd (1/s)	Affinity, KD	chi^2 global fit
2H2 murine IgG2b	6.10 E+04	2.01 E-03	33nM	0.902
2H2 scAb	4.91 E+04	1.91 E-03	39nM	0.429
2H2 IgG chimera	1.10 E+05	2.73 E-03	25nM	0.295

Example 12: ORF0657n Based Sequences

The highlighted amino acids (indicated by bold and underlying) present in SEQ ID NOs: 4-19 show amino acid alterations to ORF0657n:

0657n (SEQ ID NO: 1)

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKAVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNSRPIDFE
MKKENGEQQFYHYASSVKPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFSVSNGT
KAVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLKA
EYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

0657nC/e (SEQ ID NO: 2)

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKAVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNSRPIDFE
MKKENGEQQFYHYASSVKPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFSVSNGT
KAVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLKA
EYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKNLEHHHHHH

0657nH/y (SEQ ID NO: 3)

MAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVANAVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKA
TNNTYPILNQELREAIKNPAIKDKDHSAPNSRPIDFEMKKKDGTQQFYHYASSVKPARVIFTDSKPEIELGLQSGQF
WRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFSVSNGTKAVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEE
DYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLKAEYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDT
KYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVMETTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKT
LYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKSNKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDD
NKQLPSVEKENDASSESGKDKTPATKPTKGEVESSSTTPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSA
PLQKANIKNTNDGHTQSQNNKNTQENKAKS

SEQ ID NO: 4

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNSRPIDFE
MKKKDGTQQFYHYASSVKPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFSVSNGT
KEVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLKA
EYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 5

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNWRPIDFE
MKKKDGTQQFYHYASSVEPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFSVSNGT
KEVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLKA
EYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 6

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNWRPIDFE
MKKKDGTQQFYHYASSVEPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFSVSNGT
KAVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELEKIQDKLPEKLKA
EYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 7

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNWRPIDFE
MKKKDGTQQFYHYASSVEPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFSVSNGT
KEVKIVSSTHFNNKEEKYDYTLMVFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLKA
EYKKKLEDTKKALAEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 8

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNWRPIDFE
MKKKDGTQQFYHYASSVEPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFSVSNGT
KEVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELEKIQDKLPEKLKA
EYKKKLEDTKKALAEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 9

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNWRPIDFE
MKKKDGTQQFYHYASSVEPARVIFTKSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTDKDYAYIRFSVSNGT
KEVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELEKIQDKLPEKLKA
EYKKKLEDTKKALAEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 10

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNWRPIDFE
MKKKDGTQQFYHYASSVEPARVIFTKSKPEIELGLQSGSTWRKFEVYEGDKKLPIKLVSYDTDKDYAYIRFSVSNGT
KEVKIVSSTHFNNKEEKYDYTLMVFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELEKIQDKLPEKLKA
EYKKKLEDTKKALAEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST

TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 11

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AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNWRPIDFE
MKNDKGTQQFYHYASSVEPARVIFTKSKPIIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTDKDYAYIRFSVSNGT
KEVKIVSSTHFNNKEEKYDYTLMVFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELEKIQDKLPEKLKA
EYKKKLEDTKKALAEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 12

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNWRPIDFE
MKNDKGTQQFYHYASSVEPARVIFTKSKPIIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTDKDYAYIRFSVSNGT
KEVKIVSSTHFNNKEEKYDYTLMVFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELEKIQDKLPEKLKA
EYKKKLEQTKKALAEQVKSAITEFQNVQPTNEKMTDLQDAHYVVYESVENSESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 13

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
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MKNDKGTQQFYHYASSVEPARVIFTKSKPIIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTDKDYAYIRFSVSNGT
KEVKIVSSTHFNNKEEKYDYTLMVFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELEKIQDKLPEKLKA
EYKKKLEQTKKALAEQVKSAITEFQNVQPTNEKMTDLQDAHYVVYESVENSESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGKRVRTISKDAKNNTRTIIFPYVEGKALYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 14

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MKKKDGTQQFYHYASSVKPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPVKLVSYDTVKDYAYIRFSVSNGT
KAVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKLQEKLPEKLKA
EYKKKLEDTKKALDEQVKSAVTEFQNVQPTNDKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME

TTNDDYWKDFMVEGQSVRTISKDAKNNTRTIIFPYIEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 15

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MKKKDGTQQFYHYASTVKPARVIFTDTKPEIELGLQSGQFWRKFEVYEGDKKLPVKLVSYDSVKDYAYIRFSVSNGT
RAVKIVSSTHYNNKEEKYDYTLMEFAQPIYNSADKYKTEEDYKAEKLLAPYKKAKTLERQVYELNKLQDKLPEKLKA
EYKKKLDDTKKALDDQVKSAVTEFQNVQPTNEKMTDLQDTKYVVFESVENNESVMDTFVKHPIKTGMLNGKKYVVME
TTNDDYWKDFIVEGQRVRTVSKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 16

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MKKKDGTQQFYHYASSVKPARVIFTDSGPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFPVSNGT
KAVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLKA
EYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 17

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AVSVSNKEVEAPTSETKEAKEVKEVKAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIIDKDHSAPNSRPIDFE
MKKKDGTQQFYHYASSVKPARVIFTDSGPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFPVSNGT
KAVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLKA
EYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESEENNESMMDTFVKHPIYTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 18

MNKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGGTNTEAQPKTEAVASPTTTSEKAPETKPVAN
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MKKKDGTQQFYHYASSVKPARVIFTDSGPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFPVSNGT
KAVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKDEEDYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLKA
EYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESEENNESMMDTFVKHPIYTGMLNGKKYMVME
TTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDKEAFTKANTDKS
NKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVESSST
TPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLPQTGEE
SNKDMTLPLMALLALSSIVAFVLPRKRKN

SEQ ID NO: 19

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DFEMKKKDGTQQFYHYASSVKPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYDTVKDYAYIRFSVS
NGTKAVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEK
LKAEYKKKLEDTKKALDEQVKSAITEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYM
VMETTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDYDGQYHVRIVDVDKEAFTKA
NTDKSNKKEQQDNSAKKEATPATPSKPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEV
ESSSTTPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQENKAKSLP
QTGEESNKDMTLPLMALLALSSIVAFVLPRKRKN

Other embodiments are within the following claims. While several embodiments have been shown and described, various modifications may be made without departing from the spirit and scope of the present invention.

WHAT IS CLAIMED IS:

1. An isolated antigen binding protein comprising a first variable region and a second variable region, wherein said binding protein binds to a target region selected from the group consisting of: mAb IG3.BD4 target region, mAb 2H2.BE11 target region, mAb 13C7.BC1, and mAb 13G11.BF3 target region.

2. The binding protein of claim 1, wherein said target region is the mAb 2H2.BE11 target region and said first variable region is a V_h region comprising:

a first V_h CDR comprising amino acids 36-45 of SEQ ID NO: 20 or a sequence differing from amino acids 36-45 by one amino acid;

a second V_h CDR comprising amino acids 50-65 of SEQ ID NO: 20 or a sequence differing from amino acids 50-65 by one amino acid; and

a third V_h CDR comprising amino acids 98-107 of SEQ ID NO: 20 or a sequence differing from amino acids 98-107 by one amino acid.

3. The binding protein of claim 2, wherein said second variable region is a V_l region comprising:

a first V_l CDR comprising amino acids 24-33 of SEQ ID NO: 21 or a sequence differing from amino acids 24-33 by one amino acid;

a second V_l CDR comprising amino acids 49-55 of SEQ ID NO: 21 or a sequence differing from amino acids 49-55 by one amino acid; and

a third V_l CDR comprising amino acids 88-96 of SEQ ID NO: 21 or a sequence differing from amino acids 88-96 by one amino acid.

4. The binding protein of claim 3, wherein said binding protein is an antibody.

5. The binding protein of claim 4, wherein said antibody is a monoclonal antibody.

6. The binding protein of claim 4, wherein said V_h region is either SEQ ID NO: 20, a humanized SEQ ID NO: 20 , or a de-immunized SEQ ID NO: 20; and said V_l region is either SEQ ID NO: 21, a humanized SEQ ID NO: 21, or a de-immunized SEQ ID NO: 21.

7. The binding protein of claim 6, wherein said binding protein is an antibody comprising (a) a heavy chain comprising said V_h region, and a human hinge, CH_1, CH_2, and CH_3 regions from an IgG_1, IgG_2, IgG_3 or IgG_4 subtype; and (b) a light chain comprising said V_l region, and either a human kappa C_L or human lambda C_L.

8. The binding protein of claim 3, wherein

said V_h region comprises said first V_h CDR consisting of amino acids 36-45 of SEQ ID NO: 20, said second V_h CDR consisting of amino acids 50-65 of SEQ ID NO: 20, and said third V_h CDR consisting of amino acids 98-107 of SEQ ID NO: 20 and;

said first V_l region comprises said first V_l CDR consisting of amino acids 24-33 of SEQ ID NO: 21, said second V_l CDR consisting of amino acids 49-55 of SEQ ID NO: 21, and said third V_l CDR consisting of amino acids 88-96 of SEQ ID NO: 21.

9. The binding protein of claim 8, wherein said binding protein is an antibody.

10. The binding protein of claim 9, wherein said antibody is a monoclonal antibody.

11. The binding protein of claim 9, wherein said V_h region is either SEQ ID NO: 20, a humanized SEQ ID NO: 20, or a de-immunized SEQ ID NO: 20; and said V_l region is either SEQ ID NO: 21, a humanized SEQ ID NO: 21, or a de-immunized SEQ ID NO: 21.

12. The binding protein of claim 8, wherein said binding protein is an antibody comprising (a) a heavy chain comprising said V_h region, and a human hinge, CH_1, CH_2, and CH_3 regions from an IgG_1, IgG_2, IgG_3 or IgG_4 subtype; and (b) a light chain comprising said V_l region, and either a human kappa C_L or human lambda C_L.

13. The binding protein of claim 12, wherein said heavy chain consists essentially of the amino acid sequence of SEQ ID NO: 22; and said light chain consists essentially of the amino acid sequence of SEQ ID NO: 23.

14. A nucleic acid comprising a recombinant gene comprising a nucleotide sequence encoding an antibody variable region that binds to a target region selected from the group consisting of: mAb IG3.BD4 target region, mAb 2H2.BE11 target region, mAb 13C7.BC1, and mAb 13G11.BF3 target region.

15. The nucleic acid of claim 14, wherein said target region is the mAb 2H2.BE11 target region and said variable region is a V_h region comprising:

a first V_h CDR comprising amino acids 36-45 of SEQ ID NO: 20 or a sequence differing from amino acids 36-45 by one amino acid;

a second V_h CDR comprising amino acids 50-65 of SEQ ID NO: 20 or a sequence differing from amino acids 50-65 by one amino acids; and

a third V_h CDR comprising amino acids 98-107 of SEQ ID NO: 20 or a sequence differing from amino acids 98-107 by one amino acid.

16. The nucleic acid of claim 15, wherein said V_h region comprises said first V_h CDR consisting of amino acids 36-45 of SEQ ID NO: 20; said second V_h CDR consisting of amino acids 50-65 of SEQ ID NO: 20; and said third V_h CDR consisting of amino acids 98-107 of SEQ ID NO: 20.

17. The nucleic acid of claim 14, wherein said variable region is either SEQ ID NO: 20, a humanized SEQ ID NO: 20, or a de-immunized SEQ ID NO: 20.

18. The nucleic acid of claim 17, wherein said recombinant gene encodes an antibody heavy chain comprising said variable region, a human hinge, and CH_1, CH_2, and CH_3 regions from an IgG_1, IgG_2, IgG_3 or IgG_4 subtype.

19. The nucleic acid of claim 14, wherein said variable region is a V_l region comprising:

a first V_l CDR comprising amino acids 24-33 of SEQ ID NO: 21 or a sequence differing from amino acids 24-33 by one amino acid;

a second V_l CDR comprising amino acids 49-55 of SEQ ID NO: 21 or a sequence differing from amino acids 49-55 by one amino acid; and

a third V_l CDR comprising amino acids 88-96 of SEQ ID NO: 21 or a sequence differing from amino acids 88-96 by one amino acid.

20. The nucleic acid of claim 19, wherein said first V_l region comprises said first V_l CDR consisting of amino acids 24-33 of SEQ ID NO: 21, said second V_l CDR consisting of amino acids 49-55 of SEQ ID NO: 21, and said third V_l CDR consisting of amino acids 88-96 of SEQ ID NO: 21.

21. The nucleic acid of claim 14, wherein said variable region is either SEQ ID NO: 21, a humanized SEQ ID NO: 21, or a de-immunized SEQ ID NO: 21.

22. The nucleic acid of claim 17, wherein said recombinant gene encodes an antibody light chain comprising said variable region and a human kappa or lambda C_L.

23. A recombinant cell comprising one or more nucleic acids of any one of claims 14-22.

24. The recombinant cell of claim 23, wherein said cell comprises both the nucleic acid of claim 18 and the nucleic acid of claim 22.

25. A method of producing protein comprising an antibody variable region comprising the steps of:

a) growing the recombinant cell of claim 23 under conditions wherein said protein is expressed; and

b) purifying said protein.

26. A method of producing protein comprising an antibody variable region comprising the steps of:

a) growing the recombinant cell of claim 24 under conditions wherein said protein is expressed; and

b) purifying said protein.

27. A pharmaceutical composition comprising the binding protein of any one of claims 1-13 and a pharmaceutically acceptable carrier.

28. A method of detecting the presence of an OFR0657n antigen in a solution or on a cell comprising the steps of: (a) providing the binding protein of any one of claims 1-13 to said solution or said cell; and (b) measuring the ability of said binding protein to bind to said antigen present in said solution or to said cell.

29. A method of treating against an *S. aureus* infection in a patient comprising the step of administering to said patient an effective amount of the binding protein of any one of claims 1-13.

30. The method of claim 29, wherein said antigen binding protein is administered in conjunction with surgery or a foreign body implant.

31. A cell line producing a protein that is either mAb 1G3.B3, mAb 2H2.B8, mAb 13C7.D12, or mAb 13G11.C11, or that competes with either mAb IG3.B3., mAb 2H2.B8, mAb 13C7.D12, or mAb 13G11.C11 for binding to ORF0657n.

32. The cell line of claim 31, wherein said line is either ATCC No: PTA-7124, ATCC No: PTA-7125, ATCC No: PTA-7126 or ATCC No: PTA-7127.

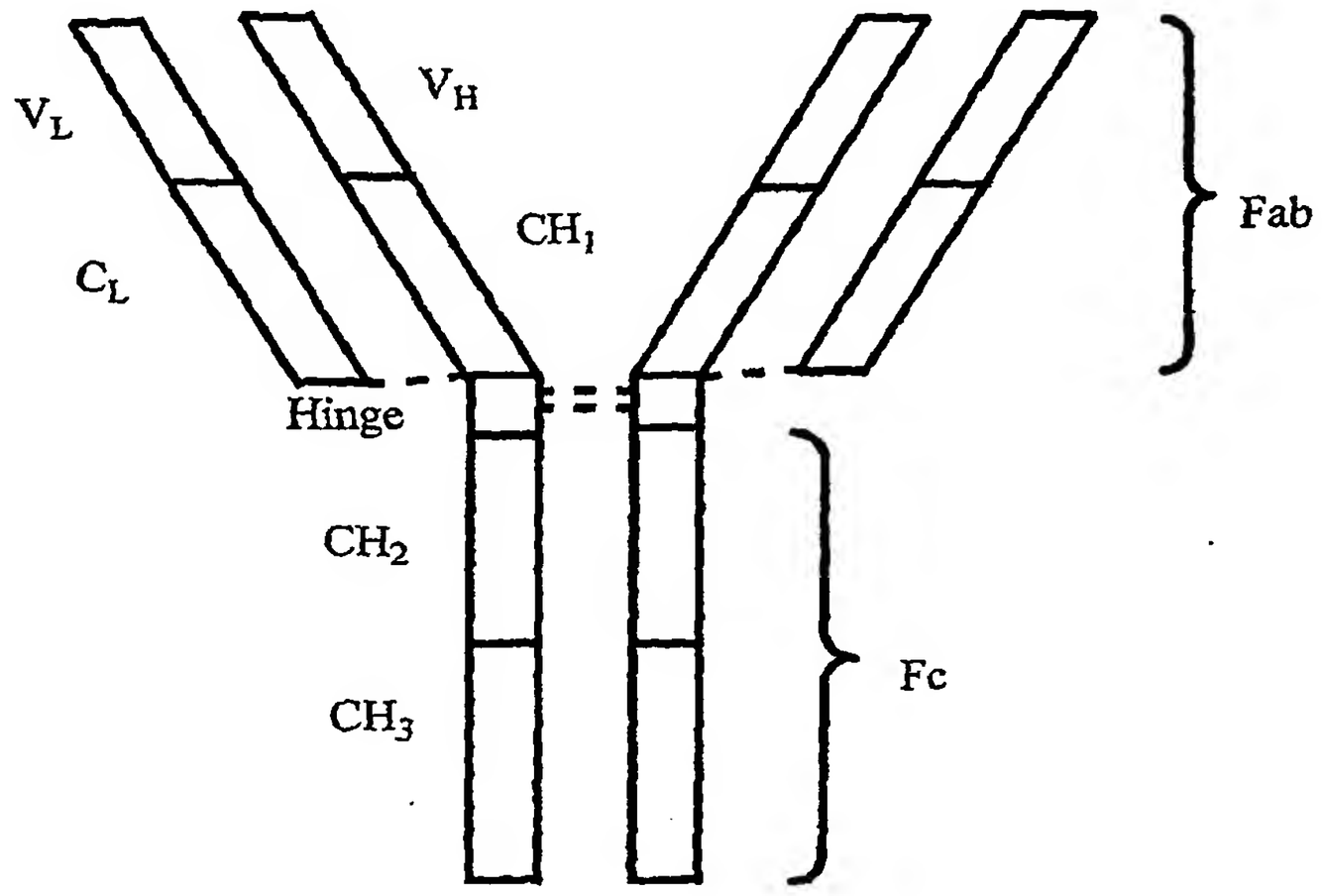

FIG. 1

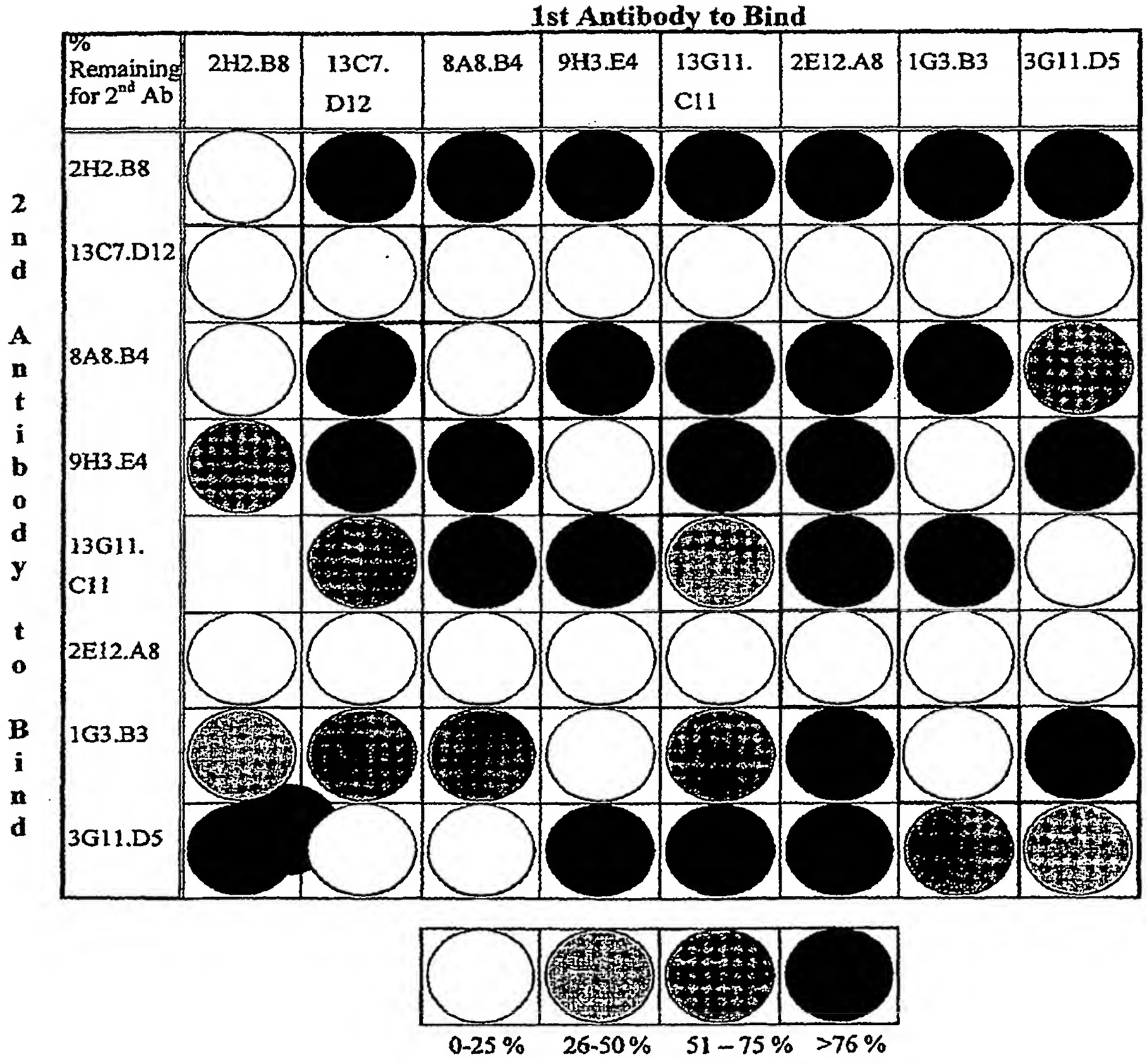
1st Antibody to Bind
% Remaining for 2nd Ab
2H2.B8
13C7. D12
8A8.B4
9H3.E4
13G11. C11
2E12.A8
1G3.B3
3G11.D5
2nd Antibody to Bind
0-25 %
26-50 %
51 – 75 %
>76 %
% Percent of Epitope Avaliable for 2nd Antibody

FIG. 2

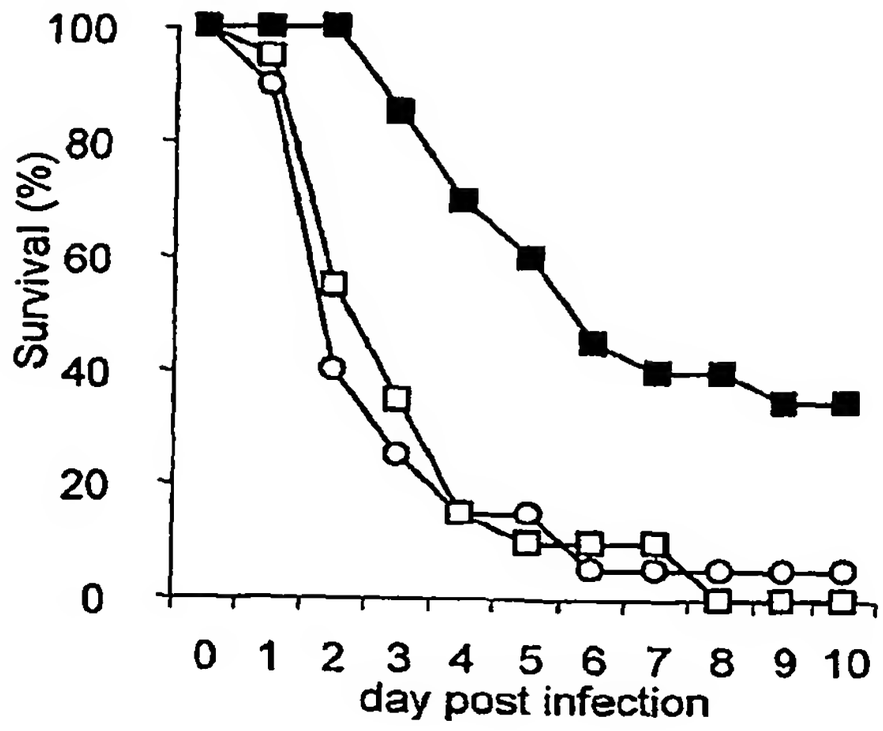

FIG. 3A

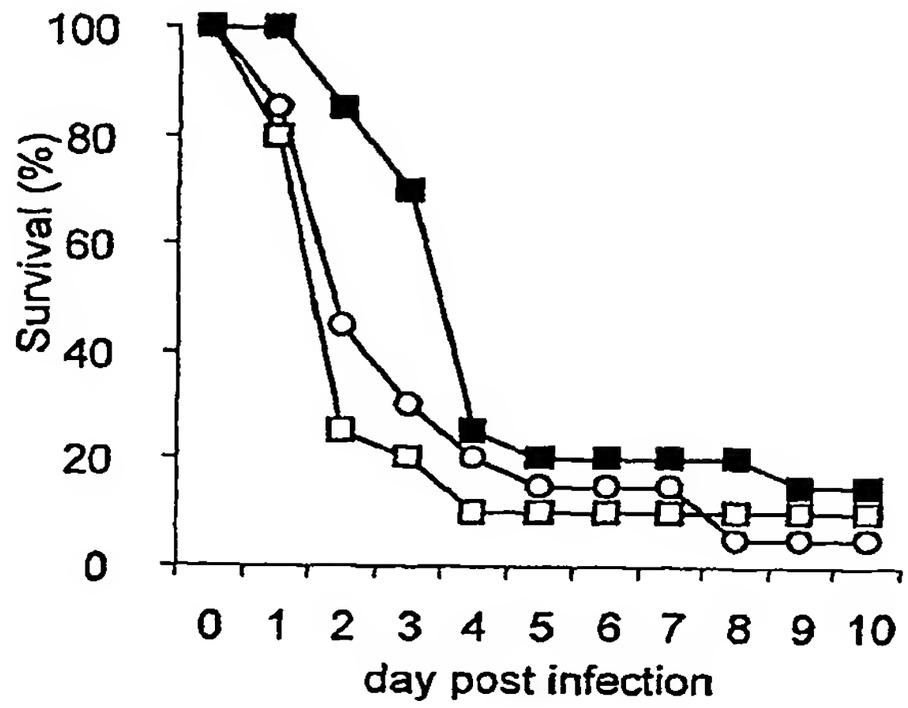

FIG. 3B

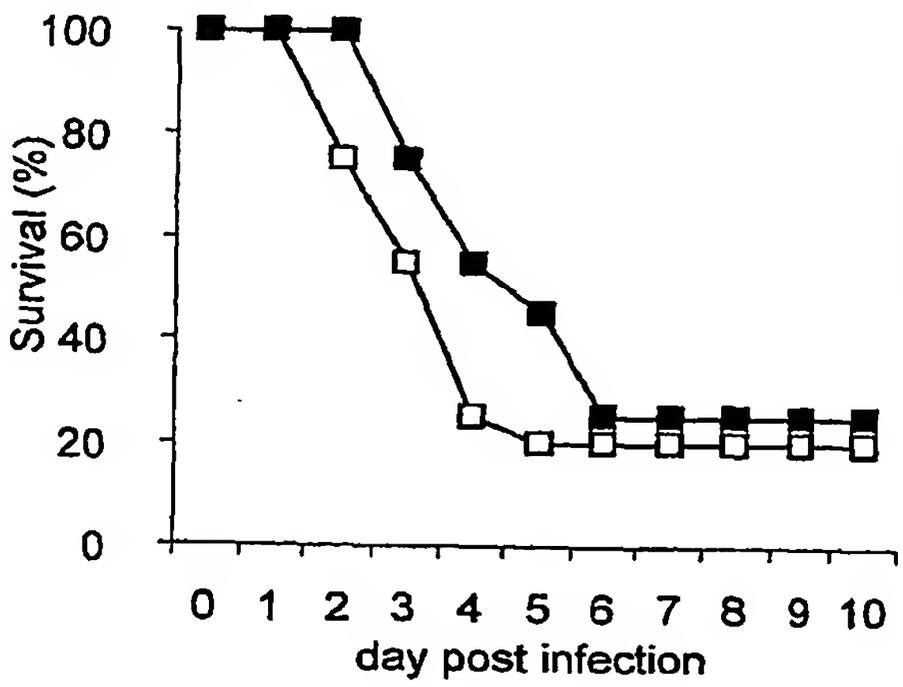

FIG. 3C

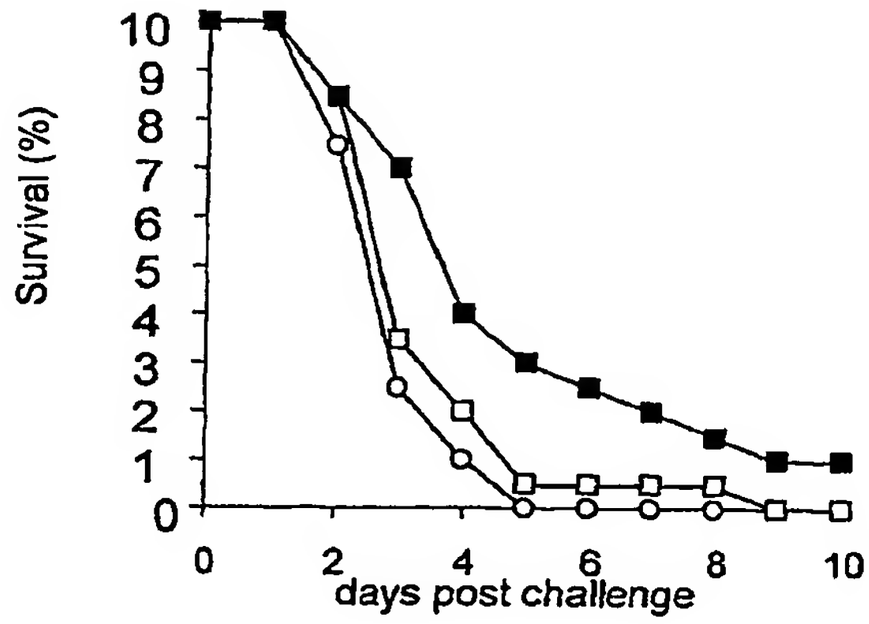

FIG. 4A

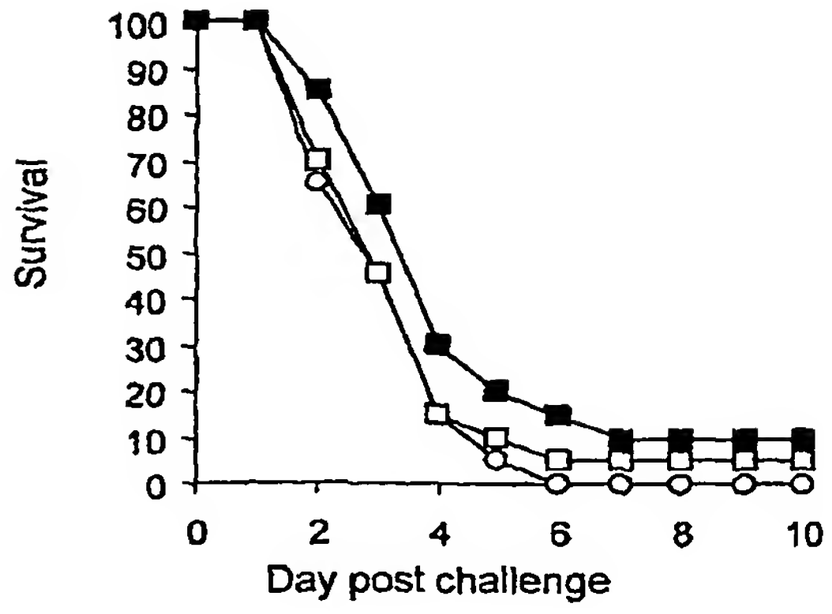

FIG. 4B

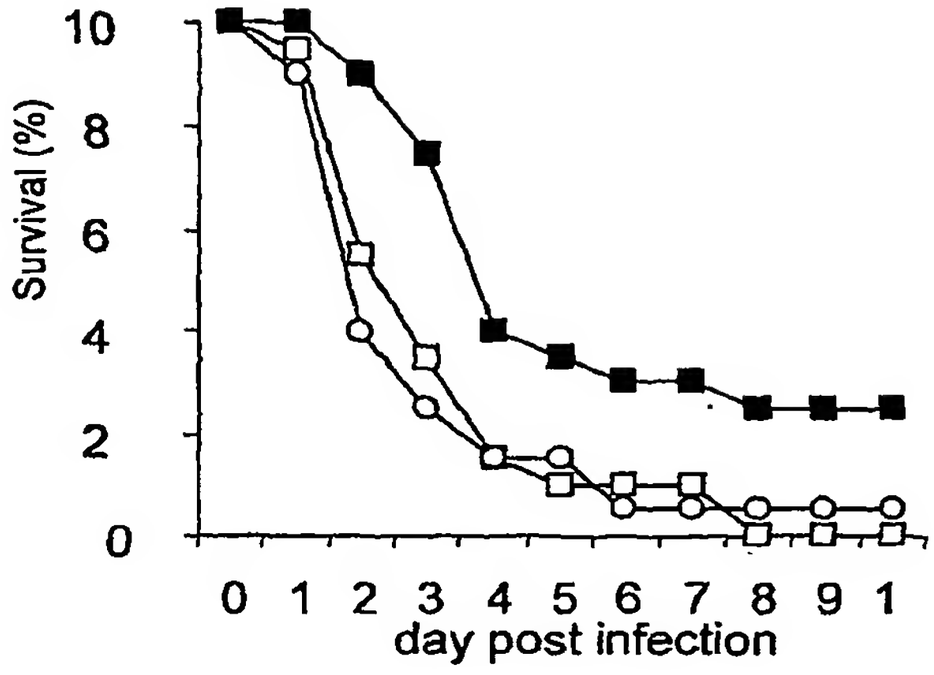

FIG. 5A

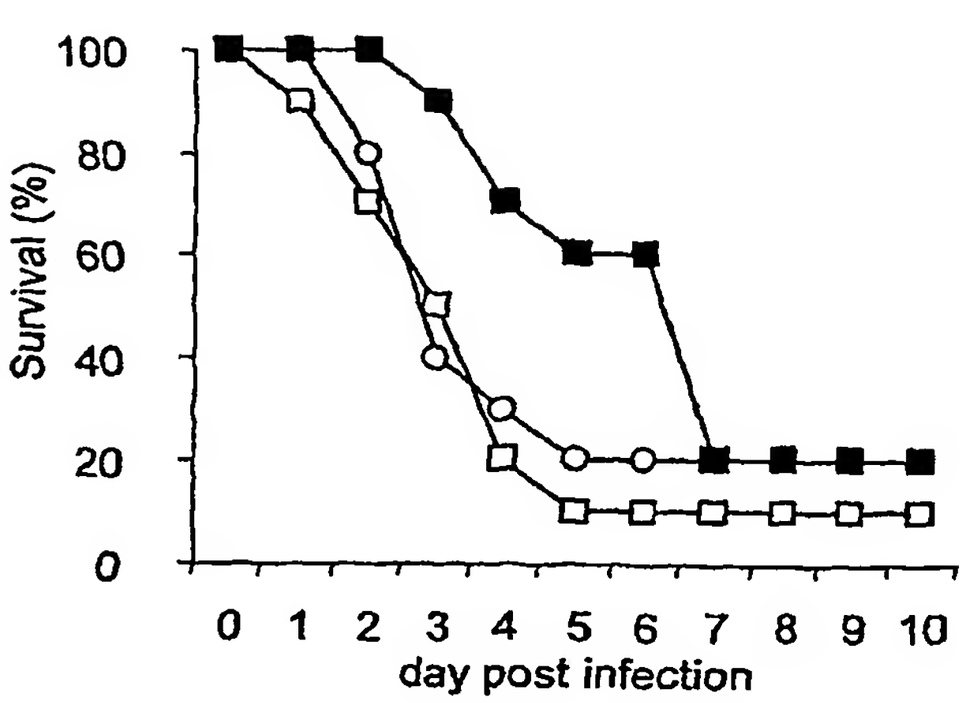

FIG. 5B

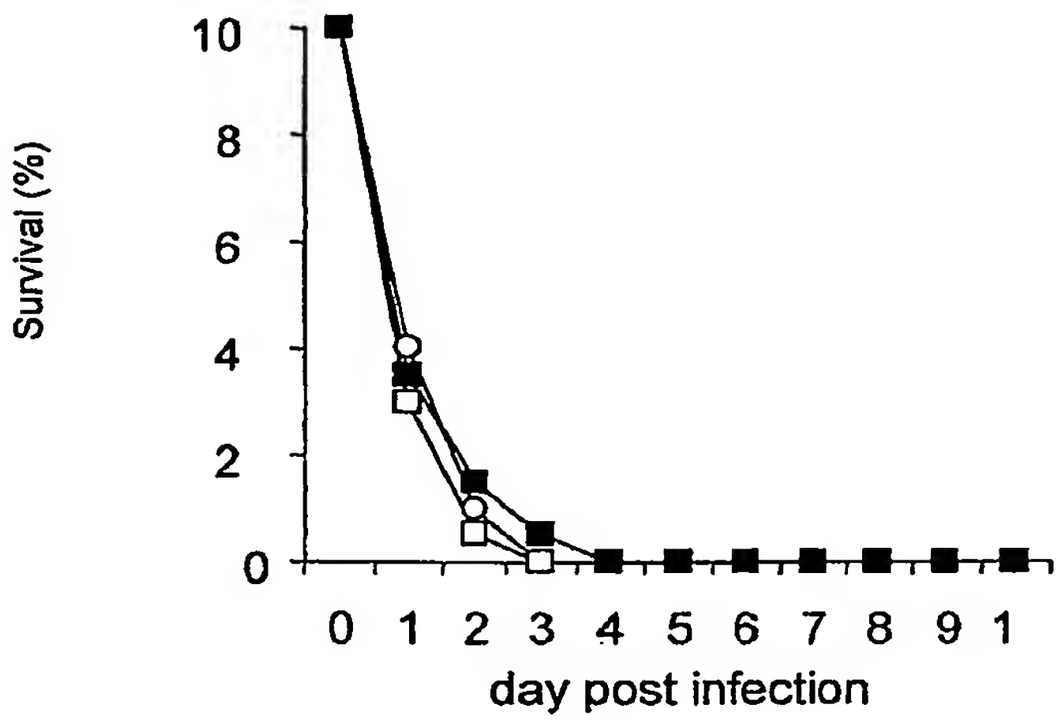

FIG. 5C